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DIURNAL AND SAMPLING VARIATIONS IN THE DETERMINATION OF HAEMOGLOBIN

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(*Received* 14 *April* 1944)

When repeated determinations of haemoglobin are made on an individual animal or man, variations of as much as 20–30% may be found during the course of the day. Since such variations undoubtedly lie outside the range to be expected from the error of replicate determinations, they are assumed to be diurnal in character (Dreyer, Bazett & Pierce, 1920; Rabinowitch, 1924). A diurnal variation, however, would be expected to be regular and recurrent, yet the recorded results show haphazard and irregular alterations. These might be the result of variability in repeated sampling of an individual's blood, independent of any diurnal variation and due to technical factors in taking the blood and to physiological alterations in the composition of the blood which are not necessarily diurnal. It is possible to distinguish this sampling variability from a true diurnal variation by an appropriate analysis of the results of repeated haemoglobin determinations. The method is the analysis of variance as developed by Fisher (1941, 1942), and this paper illustrates the value of the method when applied to the results obtained by McCarthy & Van Slyke (1939) and by others.

If haemoglobin determinations are made at a number of different times of day and on a number of different individuals, the variability of the results will be due in part to differences between individuals, in part to differences in time of day when the blood samples are taken, and in part to the remaining causes of variation which make up the natural variability or uncontrolled error of the observations. The relative magnitudes of the mathematical expressions for the three parts into which the variability may be analysed make it possible to decide whether there are real diurnal differences. The details of the arithmetical work will not be described here as they are fully treated by Fisher. In this paper the conventional probability level $P=1/20$ or 0·05 has been taken as the border line between significant and non-significant differences (Fisher).

The most carefully carried out observations on diurnal variations in haemoglobin seem to be those by McCarthy & Van Slyke (1939), who used the carbon-monoxide capacity method of Van Slyke & Hiller (1928). This is probably the most accurate method of haemoglobin estimation there is. Earlier work was exhaustively reviewed by Smith (1931), but the results were obtained by less precise methods and are less well adapted for statistical analysis.

TABLE 1. McCarthy & Van Slyke's results on twelve normal subjects in ml. of carbon monoxide capacity per 100 ml. of blood

9 a.m.	11 a.m.	2 p.m.	5 p.m.	8 p.m.	11 p.m.
19·97	19·74	19·66	19·55	19·18	19·13

Difference between 9 a.m. and 11 p.m. means $=0{\cdot}84 \pm 0{\cdot}18$ (see Table 2).
Difference between 9 a.m. and 5 p.m. means $=0{\cdot}42$.

THE RESULTS OF MCCARTHY AND VAN SLYKE

Full details of the observations are given in the original paper. The highest and lowest measurements on any one individual occurred at almost any time of day and differed on the average by 1·3 vol. CO, or 7% haemoglobin. Throughout this paper 100% haemoglobin has been assumed to equal 18·5 vol. of gas-combining capacity, as on Haldane's scale. The complete table of results has had to be split into two for statistical analysis, since five individuals were examined on 2 days each, and thirteen on 1 day only. Of these last, one 11 p.m. result is missing, so that the complete results on the remaining twelve will be first considered. The average figures for each of the sampling times are given in Table 1 and show a steady, if small, decline through the day. The analysis of variance is set out in Table 2 and the diurnal variation in blood carbon-monoxide capacity between 9 a.m. and 11 p.m. is found to be highly significant when compared with the uncontrolled error of the observations.

TABLE 2. Analysis of variance of results on twelve subjects by McCarthy & Van Slyke

9 a.m.–11 p.m.	Degrees of freedom	Variance	Variance ratio	Probability (P)
Individual differences	11	6·1201	—	—
Diurnal differences	5	1·2824	6·2	Less than 0·001
Uncontrolled error	55	0·2071	—	—

Standard deviation of repeated observations $=\sqrt{0{\cdot}2071}=0{\cdot}46$ vol. CO.
Standard error of difference between two means each derived from twelve observations

$$=\sqrt{\frac{2\times 0{\cdot}2071}{12}}=0{\cdot}18 \text{ vol. CO.}$$

It is also worth while to examine the smaller changes between 9 a.m. and 5 p.m., since these are the limits of the ordinary working day in laboratory and hospital. An exactly similar variance analysis (not given here) yields a value of P greater than 0·05 so that the diurnal variation over this shorter

period is not statistically significant: nor does it become significant with the increase in the number of observations which results from including in the analysis the measurements of the five subjects on the first of the 2 days on which they were examined. It is unfortunately not possible to include the second-day results of these five subjects, since there is a marked difference (P less than 0·05) between the uncontrolled error variances of the five first-day and five second-day sets of results due to the much smaller uncontrolled error of the latter (Table 3). This means that the first- and second-day sets

TABLE 3. Analysis of variance of results on five subjects by McCarthy & Van Slyke

	First day*		Second day	
9 a.m.–5 p.m.	Degrees of freedom	Variance	Degrees of freedom	Variance
Individual differences	4	5·7689	4	6·0635
Diurnal differences	3	0·4456	3	0·5164
Uncontrolled error	10	0·2667	12	0·0627

* The analysis is complicated by the loss of two measurements from the first-day series. The missing values have been estimated by minimizing the sum of squares for error as Fisher (1942) has suggested. This allows the utilization of the other observations on the individuals concerned, but the analysis is, of course, not as precise as if the missing value had actually been observed. The actual estimates here are 18·19 and 20·38. The difference in uncontrolled error variance between the first- and second-day series is not due to the use of estimated values.

are not comparable and should not therefore be grouped as if they were. Nevertheless, this reduction in error is important from the experimental point of view, for the smaller the uncontrolled error the smaller the diurnal difference which is significant. Without the method of variance analysis this reduction in variability in the second 5-day set of results went undetected, so that the conditions under which the sampling variability of haemoglobin measurement was brought so low, although of great interest, were not recorded.

DISCUSSION

The method of variance analysis has provided an exact measure of the random variability of the results and of the probability of the averaged diurnal variations of all the subjects having occurred as a consequence of this random variability. In the last resort the significance of any particular value of P is always a matter of opinion, but its actual value is a property of the data and is fixed by them. However strict the criterion of significance, the analysis shows that there is a real diurnal variation, even if its size is much smaller than has been supposed. Diurnal variation, defined as a regular change in haemoglobin through the day, is found from McCarthy & Van Slyke's results to be a steady decrease amounting to about 4% between 9 a.m. and 11 p.m. This is the average result obtained by eliminating differences between individuals and variability due to technical and sampling factors and thus represents a physiological normal value.

In advanced heart failure Rabinowitch & Strean (1924) made the interesting observation that there was a much smaller variability than in normal people.

It should be pointed out that it is possible that the time of day for maximum and minimum haemoglobin levels may be different for different individuals. The method of analysis used in this paper will obscure any such individual differences in diurnal variation which will, on the other hand, go to swell the uncontrolled error. It would be possible to detect such individual differences by an analysis of repeated serial estimations on several days for each individual. The duplicate series of McCarthy & Van Slyke have been shown to differ so considerably in the magnitude of the uncontrolled error that they should not be considered as a homogeneous group of data, but if they are so grouped and analysed for differences in diurnal variation between individuals no such individual differences are found. The data do not bear out the suggestion that each individual has a characteristic diurnal variation of his own, though a large enough series of repeated observations might show that this hypothesis was true.

The uncontrolled error of McCarthy & Van Slyke's observations consists of technical errors in the performance of the estimations, sampling variability and any other unspecified factors which affect the results. The variability of repeated estimations of carbon monoxide capacity on the same sample of blood is very small (Van Slyke & Hiller). It is therefore safe to assume that the uncontrolled error of McCarthy & Van Slyke's data is almost entirely due to sampling variability, with the reservation discussed in the last paragraph. The variation with repeated sampling will be due to local environmental and metabolic influences which affect the composition of the blood in the vein from which the blood is taken, and also to more general factors which alter the proportion of red cells to plasma in the circulating blood. None of these variations are necessarily diurnal although they may normally occur in a regular manner during the day in relation to such regularly recurring events as meals. Any such regularly occurring changes will have been included in the diurnal variation in the analysis and by definition.

Lastly, it is perhaps worth pointing out that if the diurnal variation is disregarded and included in the random variability, the standard deviation of repeated estimations is 0·54 vol. of carbon monoxide. On the normally accepted basis of twice the standard error, this means that there must be a difference of nearly 8% between two haemoglobin estimations on one individual before this difference can be attributed with safety to anything but experimental error. In most experimental and clinical work where haemoglobin measurements are used to assess an experimental or therapeutic procedure, there will be at least as big an experimental error as this, against which the changes in haemoglobin must be interpreted. The magnitude of

sampling and diurnal variations sets a limit, too, beyond which it becomes no longer useful to increase the accuracy of haemoglobinometer readings. The limit is reached when the standard deviation of repeated measurements is about 1% on Haldane's scale.

Summary

1. Evidence for diurnal variations in haemoglobin has been examined by the method of variance analysis.

2. In the best series of observations there was a significant fall in haemoglobin between 9 a.m. and 11 p.m. of 0·8 vol. of carbon monoxide capacity, or about 4%.

3. There was no significant diurnal change during the working day from 9 a.m. to 5 p.m.

4. The variation in haemoglobin content with repeated sampling of venous blood has a standard deviation of 0·54 vol. of carbon monoxide, or 0·46 vol. after diurnal variation has been allowed for.

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DETERMINATION OF HAEMOGLOBIN AND METHAEMOGLOBIN

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(*Received* 24 *June* 1944)

In the description of the methods for the determination of haemoglobin and methaemoglobin according to Van Slyke & Hiller (1928), it is reported that 'the carbon monoxide tension of approximately 25 mm. is sufficient to change the haemoglobin quantitatively to HbCO'.

There is no doubt that the method as described by Van Slyke & Hiller usually gives results of a high standard of accuracy, but the experiments described below show that without special precautions there is some risk that low results may be obtained with freshly drawn blood which contains carbon dioxide.

In a series of experiments in which blood was shaken with carbon monoxide for 1·5 min., a pressure of 25 mm. CO was sufficient to saturate blood which had been exposed to air, but not sufficient to saturate freshly drawn blood.

One sample of blood gave a capacity of 18·53 vol. % before exposure to air, and values of 19·4, 20·5 and 20·9 after exposure to air for varying periods. It is possible that these differences could be accounted for by the loss of carbon dioxide from blood exposed to air, since Douglas, Haldane & Haldane (1912) have shown that removal of carbon dioxide increases the affinity of blood for carbon monoxide.

Later experiments, some of which are recorded in Table 1, showed that, when the tension of carbon monoxide was increased from 25 to 100 mm. or more, there was no difference between the observed carbon monoxide capacities of untreated blood and blood exposed to air. It has been found convenient to use 10 ml. of carbon monoxide, which gives a pressure of 150 mm. instead of 2 ml. as suggested by Van Slyke & Hiller.

The observation that freshly drawn blood may give low results may be of significance for the interpretation of certain measurements of methaemoglobin in blood. Ammundsen (1939, 1941), for example, obtained relatively high values for inactive haemoglobin in a series of investigations, and concluded that relatively large amounts of methaemoglobin occurred in healthy persons. I verified her observation but found that, if an equally high carbon monoxide pressure (10 ml. of carbon monoxide, giving a pressure of about 150 mm.) was used for the determination of the active and the total haemoglobin, the

same values were obtained for both. Thus the blood of healthy persons does not contain any methaemoglobin demonstrable with the gasometric method.

TABLE 1

Untreated blood						Blood shaken in air					
	Active Hb						Active Hb				
Total Hb	2 ml. CO	10 ml. CO	$T-A_2$	$T-A_{10}$	Alk. res.	Total Hb	2 ml. CO	10 ml. CO	$T-A_2$	$T-A_{10}$	Alk. res.
20·68	17·46	20·98	3·22	−0·30	54·18	21·01	20·58	20·88	+0·43	+0·13	37·93
19·93	15·83	20·18	4·10	−0·25	58·99	19·80	19·83	20·15	−0·03	−0·35	41·50
17·99	15·03	18·25	2·96	−0·26	53·87	17·78	17·77	18·00	+0·01	−0·22	35·76
19·50	12·29	19·25	7·21	+0·25	59·45	19·42	19·32	19·32	+0·10	+0·10	42·64

T = total Hb. A_2 = active Hb when 2 ml. CO were used. A_{10} = active Hb when 10 ml. CO were used. Alk. res. = alkali reserve, vol. CO_2/100 ml. blood.

This is illustrated by the following experiment (Table 1). Venous blood was taken under paraffin. It was divided into two parts. One was kept under paraffin and the active and total haemoglobin estimated according to Van Slyke & Hiller with the use of, first, 2 ml. of carbon monoxide (25 mm.) and, secondly, 10 ml. (about 150 mm.). The other part was first exposed to air by shaking and then the same determinations done. As seen from Table 1 the same values for the total haemoglobin, within the limits of error of the method, were obtained from both specimens. In the case where only 2 ml. of carbon monoxide were used for estimating the active haemoglobin and the blood had been protected from air, a large difference was observed between total and active haemoglobin. Thus one would be inclined to believe that a large amount of inactive haemoglobin was present in this case. When the carbon monoxide pressure was raised to about 150 mm., however, no difference was seen between active and total haemoglobin. Moreover, when the blood was exposed to air before the determinations, no difference was observed between active and total haemoglobin.

SUMMARY

Under certain conditions, the method of Van Slyke & Hiller for determining carbon monoxide capacity may give low results with freshly drawn blood which contains carbon dioxide, unless the volume of carbon monoxide used for saturation is raised from 2 to 10 ml.

No methaemoglobin could be demonstrated in normal blood when 10 ml. of carbon dioxide were used for saturation.

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FORMATION OF ACETYLCHOLINE IN CELL-FREE EXTRACTS FROM BRAIN

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(*Received* 23 *August* 1944)

Synthesis of acetylcholine in brain slices and pulp was described by Quastel, Tennenbaum & Wheatley (1936) and by Stedman & Stedman (1937). Their main results have been confirmed by several workers. Synthesis of acetylcholine was shown to occur in brain which had been dried in a desiccator and powdered before use (Feldberg, 1945). In all these instances the synthesis took place aerobically and depended on the presence of some particulate matter, though not necessarily on that of intact cells.

Recently, Nachmansohn & Machado (1943) have shown that acetylcholine is formed *anaerobically* in homogenized brain tissue, provided that adenosinetriphosphate (ATP), fluoride and choline are added. They expressed the view that the acetylation of choline is catalysed by an enzyme, the 'choline acetylase'. They claim to have brought this enzyme into solution, although the evidence presented in their paper does not exclude the possibility that the enzyme might have been associated with the cell granules which abound in homogenized brain tissue.

The observations described in this paper show that the enzymic system which catalyses the formation of acetylcholine can be completely separated from the particulate matter of the brain, and that it can be obtained in the form of cell-free solutions prepared by saline extraction of the acetone-dried tissue. The use of such extracts enabled us to follow up the metabolism of ATP in the course of the synthesis of acetylcholine.

In this paper evidence is offered which shows that the connexion between the presence of ATP and the acetylation of choline is not merely restricted to anaerobic conditions, but that it also exists under aerobic conditions which resemble more closely the circumstances under which acetylcholine is formed *in vivo*.

The use of enzyme extracts prepared from acetone-dried brain powder made it possible to study the finer mechanism of the action of glucose and of Ca-

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and K-ions on the synthesis proper, without interference from any reactions associated with the release of acetylcholine. In previous studies with these substances such a distinction was hardly possible.

Methods

Rats were killed by a blow on the neck, and the brains were removed by transverse section in front of the cerebellum. The brains were either homogenized or converted into acetone-dried powder.

The tissue was homogenized with Ca-free Ringer-bicarbonate solution and diluted so that 3 ml. were equivalent to 250 mg. brain, fresh weight. To prepare the acetone powder, the brains were ground in a mortar with ice-cold acetone; the powder was collected on a small Buchner funnel and dried in air. One g. dry powder, which corresponded to between 5·5 and 6 g. fresh tissue, was ground with 50 ml. solution and centrifuged. The extracts were made with the Ca-free Ringer-bicarbonate solution or with a 'Mg-saline solution', composed of 0·9 % NaCl and 0·029 % $MgSO_4$. In some experiments Mg was omitted, in others phosphate was added to the saline solutions. These changes did not affect the main results.

The incubation was carried out at 37° C. in Thunberg tubes. The main compartment of each tube contained 3 ml. of homogenized brain or 2·5 ml. of the centrifuged extract from acetone powder. It also received the following substances (each in 0·1 ml.): 6 mg. KCl, 2–3 mg. NaF, 0·5 mg. eserine sulphate, 3 mg. choline and 1·5 mg. Na-acetate. ATP was introduced into the side bulb of the Thunberg tube. The amounts of ATP used in the experiments with homogenized brain contained from 1·2 to 1·5 mg. of the easily hydrolysable Pyro-P_7, and, in the experiments with acetone powder, from 0·2 to 0·7 mg. P_7.

The Thunberg tubes were evacuated and refilled three times with either N_2 or a gas mixture containing 5 % CO_2 and 95 % N_2 or 95 % O_2 respectively. The N_2 and the $N_2 + CO_2$ mixture were passed over a heated copper wire to remove all traces of oxygen. The contents of the main compartment and the side bulb were then mixed, and the mixtures incubated for 1 hr.

Some of the incubated samples were used for the estimation of acetylcholine; in others phosphorus was determined. For the estimation of acetylcholine the samples were treated with 1 ml. 0·33 *N* HCl, boiled for a minute or two and cooled. They were then carefully neutralized with 0·33 *N* NaOH, made up to a definite volume, and assayed on the frog's rectus abdominis muscle against acetylcholine solutions of known strength to which equivalent amounts of the same extract were added, the acetylcholine of which, however, had previously been destroyed by a brief boiling in alkaline solution. The detailed procedure has been described elsewhere (Feldberg, 1945). Usually, concentrations equivalent to 1 mg. homogenized brain or 0·1 to 0·2 mg. acetone powder per 1 ml. solution were used for the assay.

The phosphorus estimations were carried out in samples deproteinized with trichloroacetic acid. Phosphorus was estimated in the protein free filtrate by the method of Fiske & Subbarow, as modified by Lohmann & Jendrassik (1926). Two fractions were determined, the inorganic phosphate (P_0) and the fraction hydrolyzable to inorganic phosphate by 7 min. hydrolysis with *N* HCl (P_7).

Substrates used. Adenosinetriphosphate (ATP) was prepared as Ba-salt by a modified method of Lohmann (1931). Before use, the Ba-salt was converted to the Na-salt and the pH of the solution adjusted to 7. Adenosinediphosphate (ADP) was prepared from ATP by the enzymic action of myosin (Bailey, 1942). A sample of inosinetriphosphate (ITP) was kindly given to us by Dr A. Kleinzeller.

Results

Homogenized brain

The homogenized tissue had the appearance of a milky fluid. Cell debris was present, but could be removed by centrifugation. Although the supernatant fluid was free from acetylcholine, the debris contained preformed acetylcholine in an amount equivalent to 1–2μg./g. brain.

In order to examine the synthesis of acetylcholine, samples were set up containing the cell debris, the centrifuged supernatant fluid or the whole homogenized tissue, and incubated anaerobically in the presence of eserine, ATP, NaF, choline, Na-acetate and KCl. We found that the debris was unable to synthesize acetylcholine. On the other hand, both the supernatant fluid and the whole homogenized brain tissue were able to promote the synthesis. The supernatant solution was, however, more active than the whole homogenized tissue. For instance, the figure of 17·5μg./g. recorded in Exp. 1 of Table 1, was obtained with the centrifuged homogenized tissue, whereas only

TABLE 1. Synthesis of acetylcholine in centrifuged homogenized brain under anaerobic and aerobic conditions

One hr. incubation in presence of eserine, ATP, NaF, KCl, choline and Na-acetate

	μg. acetylcholine/g. brain (fresh weight)	
Exp. no.	95 % N_2 + 5 % CO_2	95 % O_2 + 5 % CO_2
1	17·5 (29·5)	3·3
2	18	1·8
3	15·5 (21)	0·5
4	9·3	2·0

Figures in brackets relate to values obtained by the method of testing as used by Nachmansohn & Machado (see text).

10·5μg./g. were synthesized by the equivalent amount of the non-centrifuged homogenized tissue. In the presence of ATP, but without the addition of either NaF or choline, the amounts of acetylcholine synthesized were much smaller. In the absence of ATP alone there was no synthesis at all. These facts are in agreement with the findings of Nachmansohn & Machado (1943). On the whole, however, our values (Table 1) were much lower than those of these authors (35–100μg./g.). This discrepancy may have been due to the following causes.

(*a*) Our procedure for homogenizing the brain tissue may not have been as effective as that of Nachmansohn and Machado.

(*b*) The enzyme connected with the synthesis of acetylcholine may have been partly inactivated in the course of the homogenizing process which was carried out in air. It will be shown later that the enzyme system under investigation is adversely affected by aerobic conditions.

(*c*) Our method of assaying the acetylcholine content of the extracts differed from that used by Nachmansohn & Machado. We determined the acetylcholine content by comparing the effect of extracts on the frog's rectus muscle with that of inactivated extracts to which a known amount of acetylcholine had been added. In this way, proper account was taken of the fact that tissue extracts, as well as choline in subthreshold doses, make the rectus muscle more sensitive to the action of acetylcholine. Nachmansohn & Machado assayed their extracts against pure acetylcholine solutions. Exps. 1 and 3 of

Table 1 show that the values obtained by our method of testing are lower than those obtained by the method of Nachmansohn & Machado.

Breakdown of ATP. We found that, even in the presence of NaF, ATP is rapidly dephosphorylated by the homogenized brain tissue, with the liberation of inorganic phosphate. The supernatant fluid from the homogenized tissue was less active, so that 15–20% ATP were left intact after 1 hr. incubation. This may explain the higher yield of acetylcholine in experiments with the supernatant fluid, as compared with those in which whole homogenized tissue was used. The breakdown of ATP was the same in the presence and in the absence of choline. ATP could not be replaced by Na-pyrophosphate in the synthesis of acetylcholine. It was also found that the pyrophosphate is stable in the homogenized brain tissue, both in the presence and in the absence of NaF.

Azide was without any inhibiting effect on the synthesis of acetylcholine.

Glucose was found greatly to inhibit the synthesis. This action of glucose coincided with a marked inhibitory effect on the rate of dephosphorylation of ATP.

If no KCl was added to the incubated samples, the yield of acetylcholine was decreased by about 50%.

The rate of acetylcholine formation was considerably reduced under aerobic conditions (Table 1).

Acetone powder from brain

The centrifuged extract obtained from the acetone powder synthesized anaerobically large amounts of acetylcholine in the presence of eserine, ATP, NaF, KCl, choline and Na-acetate. The amounts synthesized in 1 hr. by the extracts, obtained from several preparations of the acetone powder, varied from 140 to 400μg. acetylcholine per g. acetone powder, i.e. from 500 to 1430μg./g. dry material contained in the centrifuged extract. The high values obtained with extracts suggest that the enzyme system responsible for the synthesis of acetylcholine is not affected by the treatment of the brain tissue with acetone. An acetone powder kept in a desiccator in the ice-box retained the full activity for 1 month.

As a rule, the experiments were carried out at 37° C. At 20° C. the synthesis was much smaller. Thus, for instance, in a sample kept for 1 hr. at 20° C. only 65μg./g. were formed, as compared with 253μg. acetylcholine produced at 37° C.

In Fig. 1 the amounts of synthesized acetylcholine are plotted against the time of incubation. It can be seen that the rate of synthesis decreases progressively, but is still well pronounced after 2 hr. of incubation.

We have tested the effect of various concentrations of ATP on the rate of synthesis of acetylcholine, and we found that the optimal concentration was that of 0·2 mg. ATP-P_7 in a 3·5 ml. sample, containing the extract from

50 mg. acetone powder. Thus, in one series of experiments, the amounts of acetylcholine synthesized in the presence of 0, 0·01, 0·05, 0·1 and 0·2 mg. ATP-P_7 were 6, 7·5, 113, 155 and 212μg./g. respectively. Higher concentrations of ATP occasionally enhanced, but sometimes decreased the yield of acetylcholine. In all these experiments the synthesis took place in the presence of NaF. In the absence of NaF the amounts of acetylcholine formed were 25–60 % less.

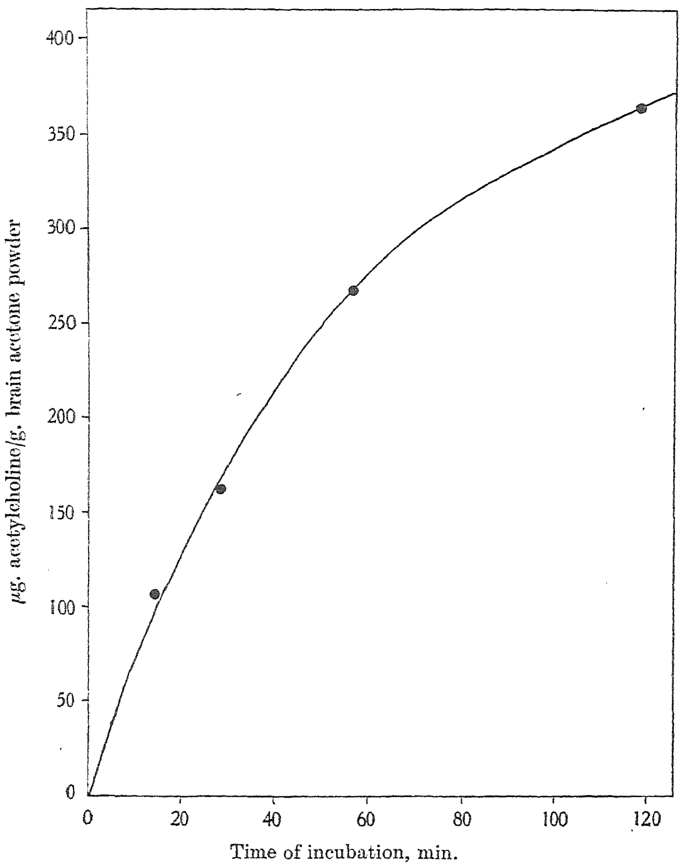

Fig. 1. Synthesis of acetylcholine in extracts made from acetone-dried brain powder.

The addition of Na-acetate was not essential for the synthesis. In the absence of choline, however, the synthesis was considerably reduced. Thus, for instance, 253μg./g. were formed in presence of both acetate and choline as compared with 250μg. of acetylcholine, formed in absence of acetate, and 41μg., formed in absence of choline.

With regard to eserine, we found that its presence was not essential, provided that the samples contained NaF. The concentration of NaF was sufficient

to abolish the activity of cholinesterase in our samples. Nevertheless, eserine was added to all samples as a routine measure.

Synthesis of acetylcholine in extracts made from the brain acetone powder was lower under aerobic than under anaerobic conditions (Table 2), although the difference was not as pronounced as in homogenized brain (Table 1).

TABLE 2. Synthesis of acetylcholine under anaerobic and aerobic conditions

One hr. incubation in presence of eserine, ATP, NaF, KCl, choline and Na-acetate

μg. acetylcholine/g. acetone powder	
Anaerobically	Aerobically
140	60
150	80
190	107
200	120
240	177
267	130

In their paper Nachmansohn & Machado expressed the opinion that the 'choline acetylase' may belong to the class of enzymes in which the active groups are the sulphydryl groups. Their view was based on the observation that iodoacetic acid, Cu-ions and iodine inhibit the synthesis of acetylcholine in the homogenized brain. Using the extracts prepared from the acetone powder, we found that 4×10^{-4} *M* Na-iodoacetate inhibited the synthesis by 80 %, whereas $2 \cdot 5 \times 10^{-6}$ *M* $CuSO_4$ inhibited the synthesis by 30 %. We have established an additional fact which supports the conception that SH— groups are essential for the synthesis of acetylcholine.

TABLE 3. The effect of sulphydryl compounds on the synthesis of acetylcholine

One hr. incubation in presence of eserine, ATP, NaF, KCl, choline and Na-acetate

Compounds added to the saline extract from 50 mg. acetone powder	μg. acetylcholine/g. acetone powder	
	Anaerobically	Aerobically
—	195	114
2 mg. reduced glutathione	230	250
2 mg. oxidized glutathione	28	35
2 mg. cysteine	340	230
4 mg. *d, l*-cystine	50	36

The experiments recorded in Table 3 show that reduced glutathione and cysteine have a powerful activating action on the aerobic synthesis of acetylcholine. Under the influence of the two SH— compounds, the synthesis of acetylcholine was brought to the same or even to a higher level than that observed anaerobically. This may be due to the fact that the SH— compounds prevent the oxidation of the SH— groups in the enzyme to inactive —SS— groups. The SH— compounds had some activating effect also on the anaerobic synthesis which suggests that some SH— groups of the enzyme might have undergone an oxidation in the course of the preparation or extraction of the acetone powder.

In contrast to the SH— compounds, the corresponding —SS— compounds, oxidized glutathione and cystine, inhibited the synthesis of acetylcholine both under aerobic and anaerobic conditions (Table 3).

The SH— compounds could not be replaced by hydroquinone nor by ascorbic acid. In fact, these two reducing agents inhibited the synthesis of acetylcholine.

TABLE 4. Liberation of inorganic phosphate from ATP

Saline extracts from 50 mg. acetone powder incubated anaerobically in presence of eserine, ATP, KCl, choline and Na-acetate

Exp. no.	Incubation hr.	NaF	μg. P_0	μg. P_7
1	0	0	290	660
	1	10^{-2} N	310	
	1	0	400	
2	0	10^{-2} N	184	468
	1	10^{-2} N	244	
	1	0	404	

Breakdown of ATP. Unlike the homogenized brain tissue, the extracts prepared from the active powder only very slowly liberated ortho-phosphate from ATP, particularly in the presence of NaF (Table 4). The rate with which free phosphate was split off remained the same in the presence and in the absence of oxygen, and was not affected by the addition of choline. Altogether no evidence was found for the existence of a relation between the synthesis of acetylcholine and the liberation of inorganic phosphate from ATP.

The role of ATP in the glycolysis of tissues is known to be that of a phosphate transferring catalyst. It was thought that ATP might act similarly in the synthesis of acetylcholine, by transferring its labile phosphate groups to an intermediary product of glycolysis, so as to produce a phosphorylated compound essential for the synthesis. To test this possibility, a number of sugars and sugar-phosphoric acid derivatives have been tested. None of these compounds, however, was active. On the contrary, both glucose and fructose (but not sucrose) exhibited, in the presence of ATP, a definite inhibitory effect on the synthesis, both under anaerobic and aerobic conditions. The same strong inhibition was observed with the Embden-ester (6-phospho-hexose), and

TABLE 5. Effect of glucose, fructose and phosphohexoses on the synthesis of acetylcholine and on the metabolism of ATP

Anaerobic incubation, 1 hr., in presence of eserine, ATP, NaF, KCl, choline and Na-acetate. Each sample contains extract from 50 mg. acetone powder and was made up to 3·3 ml.

Compounds added	μg. acetyl-choline/g. acetone powder	Phosphorus in samples	
		μg. P_0	μg. P_7
—	330	136	192
4 mg. glucose	20	117	157
4 mg. fructose	30	128	150
8·9 mg. Embden-ester (Na-salt)	18	50	165
8·3 mg. Cori-ester (K-salt)	92	28	124
10 mg. Harden-Young-ester (Na-salt)	136	—	—

a smaller effect was noticed with the Cori-ester (1-phospho-glucose). The Harden-Young-ester (1,6-diphospho-fructose) caused least inhibition (Table 5).

The determinations of phosphorus brought out two phenomena which might provide some explanation for the behaviour of these compounds: a marked decrease in the liberation of inorganic phosphate from ATP and a decrease in the ATP-P_7 value, through the formation of phosphoric acid esters which are difficult to hydrolyse. Such esterifications diminish the amount of ATP available for the synthesis of acetylcholine.

TABLE 6. Effect of varying concentrations of glucose on the synthesis of acetylcholine

Anaerobic incubation, 1 hr., in presence of eserine, ATP, NaF, KCl, choline and Na-acetate. Each sample contains extract from 50 mg. acetone powder and was made up to 3·5 ml.

Concentration of glucose mg./ml.	μg. acetylcholine/g. acetone powder With 0·2 mg. ATP-P_7	With 0·4 mg. ATP-P_7
0	250	300
0·1	44	215
0·4	22	35
1·0	23	38

Table 6 shows the effect of various concentrations of glucose on the synthesis of acetylcholine. Even with such a low concentration as 0·1 mg. glucose per ml. there was a considerable decrease in the yield of acetylcholine. However, the concentration of glucose was not the sole determining factor. The degree of inhibition by glucose varied with the concentration of ATP. With 0·2 mg. ATP-P_7 the inhibition was 76 %, with 0·4 mg. ATP-P_7 only 28 %. In the presence of reduced glutathione, glucose retained its inhibitory action on the formation of acetylcholine.

Adenosine-monophosphoric acid (muscle adenylic acid) was unable to replace ATP in either the presence, or the absence of phosphoglyceric acid. Adenosine-diphosphoric acid (ADP) was only slightly effective. 20μg. acetylcholine/g. were formed anaerobically in the presence of ADP as compared with 183μg./g., formed in the presence of the corresponding amount of ATP and with 3μg./g., formed in the absence of either of these two adenyl derivatives. Inosinetriphosphoric acid (ITP), which differs from ATP by the absence of the

TABLE 7. Synthesis of acetylcholine in presence of adenosinetriphosphate (ATP) and inosinetriphosphate (ITP)

Each sample contains extract from 50 mg. acetone powder. Anaerobic incubation, 1 hr., in presence of eserine, NaF, KCl, choline and Na-acetate

Exp. no.	μg. acetylcholine/g. acetone powder With ATP	With ITP	Without ATP or ITP	Content of ATP or ITP in μg. P_7 per each sample
1	205	28	<4	0·2
	267	28	<4	0·4
2	133	54	10	0·3
	180	83	10	0·6

amino group, was more effective than ADP but less than ATP (Table 7). This shows that the NH_2 group is also of some importance with regard to the synthesis of acetylcholine. We have investigated, therefore, whether ammonia is liberated from ATP on incubation with extracts from brain acetone powder. It was found that the deaminase activity of such extracts was small. During 1 hr., at 37° C., only 0·016 mg. NH_3-N was split off from 0·099 mg. ATP-NH_3-N under the action of 3 ml. extract made from 50 mg. acetone powder.

Aneurin. Neither aneurin nor aneurin diphosphate (cocarboxylase) could replace ATP in the synthesis of acetylcholine. In the presence of ATP aneurin inhibited the formation of acetylcholine.

TABLE 8. Effect of potassium ions on the synthesis of acetylcholine

One hr. incubation in presence of eserine, ATP, choline and Na-acetate

	μg. acetylcholine/g. acetone powder		
Exp. no.	Without KCl	With 0·02 *M* KCl	Incubation
1	127*	253	Anaerobic in presence of NaF
2	133	307	
3	120	240	
4	145	310	
5	180	360	
6	77	150	Anaerobic in absence of NaF
7	133	165	
8	120	200	
9	50	60	Anaerobic in absence of NaF, but in presence of 0·0018 *M* $CaCl_2$
10	33	42	Anaerobic in absence of NaF, but in presence of 0·0055 *M* $CaCl_2$
11	43	80	Aerobic in presence of NaF
12	70	177	

* This sample contained 0·005 *M* KCl.

The action of K and Ca ions. Potassium chloride had a stimulating effect on the synthesis of acetylcholine, both under aerobic and anaerobic conditions (Table 8). With calcium chloride, on the other hand, there was a marked inhibition (Table 9). In presence of cysteine, K^+ and Ca^{++} had similar effects. A study of the action which Ca^{++} and K^+ have on the metabolism of ATP revealed that the Ca-ions inhibit the breakdown of ATP to inorganic phosphate, and that this inhibition is not affected by the simultaneous presence of K-ions. K-ions alone did not affect the dephosphorylation of ATP to inorganic phosphate. This shows that the antagonistic action which the two ions, Ca and K, exert on the synthesis of acetylcholine is not solely dependent on the manner in which they affect the dephosphorylation of ATP. There may be yet another process connected with the synthesis of acetylcholine in which the two ions are involved. That this may be the case is suggested by the results of experiments in which the action of each of the two ions was examined separately, in the presence and in the absence of the other ion.

TABLE 9. Effect of calcium ions on the synthesis of acetylcholine
Anaerobic incubation, 1 hr., in presence of eserine, ATP, choline and Na-acetate

	μg. acetylcholine/g. acetone powder			
Exp. no.	(a) Without calcium	(b) With calcium	Molar concentration of $CaCl_2$ in (b)	Incubation
1	113	30	0·007	In absence of KCl
2	120	30	0·007	
3	140	46	0·007	
4	64	14	0·007	
5	77	33	0·0055	
6	77	50	0·0018	
7	200	40	0·007	In presence of 0·02 *M* KCl
8	130	50	0·007	
9	150	42	0·0055	
10	150	60	0·0018	

Extracts containing both Ca^{++} and K^{+} produced only a little more acetylcholine than those which had Ca^{++} alone (Exps. 9 and 10, Table 8). This shows that the inhibition caused by the Ca-ions is not reversed by K-ions. On the other hand, extracts containing both Ca^{++} and K^{+} produced much less acetylcholine than those which had K^{+} alone (Table 9). This is particularly noticeable when the yields of acetylcholine of the following samples are compared:

5 (*a*)	Without Ca^{++} and without K^{+}	77 μg.
5 (*b*)	With Ca^{++} but without K^{+}	33 μg.
9 (*a*)	Without Ca^{++} but with K^{+}	150 μg.
9 (*b*)	With Ca^{++} and with K^{+}	40 μg.

Similar differences stand out if samples 6 (*a*), 6 (*b*), 10 (*a*) and 10 (*b*) are compared. It should be pointed out that all eight samples were incubated simultaneously and contained extracts prepared from the same acetone powder.

Discussion

Since 1931, when the isolation of adenosinetriphosphate was successfully accomplished by Lohmann, there has been a continuous flow of contributions on the subject of the physiological role and metabolism of this important nucleotide. ATP has been found to react in tissues with creatine and thus to promote the formation of phosphocreatine (Lohmann, 1934). The labile groups of ATP were found to be of paramount importance in the formation and breakdown of several intermediary products of glycolysis, both in muscle (Parnas, Ostern & Mann, 1934) and in yeast (Lutwak-Mann & Mann, 1935). ATP has been recognized as the substance the breakdown of which supplies the energy of muscle contraction, and the dephosphorylation of ATP was shown to be closely associated with myosin (Engelhardt & Ljubimowa, 1939). A new and interesting feature of the action of ATP was described by Green (1943) and

by Bielschowsky & Green (1943) who identified as ATP the muscle component which causes severe shock when injected into animals.

The most recent contribution to the subject of the function of ATP comes from Nachmansohn & Machado (1943) who found that homogenized brain tissue can build up acetylcholine from choline, provided that ATP is added and the mixture is incubated anaerobically. This we were able to confirm. At the same time, however, we noticed that very little acetylcholine was formed in the presence of ATP, if the mixtures were incubated aerobically, and yet it is well known that the formation of acetylcholine in brain slices and pulp occurs in the presence of oxygen. This discrepancy remained to be explained.

In our experiments we used mainly saline extracts prepared from acetone-dried and powdered brains. Their use made it possible to demonstrate the essential role of ATP in the aerobic synthesis of acetylcholine and, moreover, that a definite function in this process must be assigned to sulphydryl compounds. The function of ATP appears to be specific. Even closely related derivatives such as adenosinediphosphate or inosinetriphosphate are but poor substitutes for ATP in the synthesis of acetylcholine. It is too early to state precisely which part of the ATP molecule is involved in the formation of acetylcholine. The evidence available so far, suggests that the most likely change is the conversion of ATP to ADP.

It has been observed that glucose, fructose and also some phospho-hexoses significantly decrease the synthesis of acetylcholine. This we attribute to esterification taking place between ATP and the various sugar compounds, with the result that less ATP remains available for the synthesis of acetylcholine.

It is interesting to note that low as well as high concentrations of glucose are capable of inhibiting the formation of acetylcholine. In this respect the results differ from those obtained with brain slices and pulp, where glucose acts as an inhibitor, only if present in high concentrations (Feldberg, 1945), whereas low concentrations stimulate and even appear to be essential for the synthesis (Mann, Tennenbaum & Quastel, 1939). Such differences, however, are not surprising if we bear in mind the fact that the conditions under which acetylcholine is synthesized in the brain tissue differ substantially from those which prevail in the cell-free extracts. For instance, whereas in the brain tissue the formation of acetylcholine is closely linked with its release, no release is possible in the extracts. In addition, the formation of acetylcholine in the brain tissue, unlike that in the extracts, occurs under conditions where respiration is a dominant factor. The inhibitory effect of glucose on the formation of acetylcholine in the extracts has been shown to be due primarily to an enzymic esterification of the labile phosphate groups of ATP. There is, as yet, no evidence that a similar mechanism is involved in the inhibitory action of glucose on the formation of acetylcholine in the brain tissue.

It has been shown previously that K-ions enhance, and Ca-ions diminish the formation of acetylcholine (Mann *et al.* 1939; Feldberg, 1945). Nachmansohn & Machado failed to observe the stimulating effect of K-ions on the formation of acetylcholine in homogenized brain and stated that the previously observed effect of K^+ was not connected directly with the formation of acetylcholine, but was due to certain changes in cell permeability. Our experiments, however, show that K-ions have a strong stimulating effect on the formation of acetylcholine both in homogenized brain and in extracts from acetone powder. The solutions used by Nachmansohn & Machado apparently already contained K-ions in a concentration sufficient to stimulate the formation of acetylcholine.

The fact that K-ions are involved in the formation of acetylcholine is of special interest in connexion with the role which K^+ plays in the mechanism of chemical transmission of nerve impulses by acetylcholine. The release of acetylcholine from cholinergic nerve fibres is generally regarded as the result of 'a mobilization of K-ions' which occurs during the passage of a nerve impulse. The release is followed by a resynthesis of acetylcholine which is said to be independent of the passage of impulses. Our results indicate that the 'mobilization of K-ions' is not only connected with the release, but that it also directly affects the resynthesis.

Summary

1. The observation of Nachmansohn & Machado (1943), that homogenized brain tissue synthesizes acetylcholine anaerobically in the presence of adenosinetriphosphate (ATP) and choline, has been confirmed. Scarcely any acetylcholine was formed under aerobic conditions.

2. The enzyme system which catalyses the formation of acetylcholine has been separated from the particulate matter of brain and obtained in the form of cell-free solutions, prepared by saline extraction of acetone-dried and powdered brain (rat).

3. The formation of acetylcholine in such extracts was better in anaerobic than in aerobic conditions. In 1 hr., at 37° C., 140–400μg. acetylcholine was formed anaerobically by the extract obtained from 1 g. acetone powder, but only half this amount was produced aerobically.

4. Reduced glutathione and cysteine strongly activated the aerobic formation of acetylcholine. On the other hand, oxidized glutathione and cysteine inhibited both the aerobic and the anaerobic formation of acetylcholine. Thus adenosinetriphosphate and SH— compounds are both essential for the aerobic formation of acetylcholine.

5. The breakdown of ATP in the extract from the brain acetone-powder has been followed in the course of the synthesis of acetylcholine. ATP could not be replaced by either adenosinediphosphate, inosinetriphosphate or adenylate.

6. The formation of acetylcholine was inhibited in the presence of glucose, fructose and certain phosphohexoses. The effect of sugars was found to be due to an enzymic esterification of the labile phosphate groups of ATP.

7. The formation of acetylcholine was enhanced by K-ions and diminished by Ca-ions.

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THE EFFECT OF ADRENALECTOMY ON THE CONTRACTILE POWER OF SKELETAL MUSCLE

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Workers investigating patients suffering from myasthenia gravis have often made extensive use of certain analogies between the clinical manifestations of the disease and the action of a number of drugs in animals and in man. Inferences drawn from comparisons of this type are always open to serious objections. Experimental work on the cause of myasthenia gravis is still hampered by the failure to induce the myasthenic syndrome in animals, and reports claiming the experimental production of typical myasthenia gravis in animals are bound to be of considerable academic and clinical interest.

Many workers favour the view that there is some close connexion between persistence of the thymus and myasthenia gravis (McEachern, 1943; Gammon, Harvey & Masland, 1941; Blalock, Mason, Morgan & Riven, 1939; Blalock, Harvey, Ford & Lilienthal, 1941; Carson, 1943). Adler (1939), however, states that the weakness of muscular performance and the rapid muscular fatigue in dogs after double adrenalectomy is indistinguishable from true myasthenia gravis and that, in his experiments, the 'myasthenic muscle' was restored to normal function on intravenous administration of 'prostigmine' (dimethyl carbamic ester of *m*-hydroxyphenyl trimethyl ammonium methyl sulphate).

In Adler's experiments the gastrocnemius muscle, in dogs under morphine-ether anaesthesia, was rhythmically stimulated with a tetanizing faradic current at a rate of 40–50 sec. The periods of stimulation lasted 0·8 sec. and were separated by rest intervals of 0·2 sec. In experiments on normal dogs such stimulation of the gastrocnemius carried on for several hours did not produce any appreciable degree of fatigue; however, when tested 5–7 hr. after double adrenalectomy, the muscle became unresponsive to direct tetanizing stimulation in a matter of a few minutes; intravenous injection of '2 c.c. of prostigmine' restored the reactivity of the muscle to tetanizing stimulation and completely abolished the 'myasthenic' symptoms.

Adler argued that the 'adynamia' of the adrenalectomized animals was 'genuine myasthenia' and was due to the removal of the inhibitory action of the adrenal cortex on the thymus gland.

It will be noted that the exact concentration of prostigmine required to produce an anti-fatigue effect in these experiments was not stated, and no analysis of the observations was attempted. Moreover there were no records of the levels of the arterial blood pressure during the various stages of the experiments, though this may be a factor of some importance since the animals died 6–8 hr. after the extirpation of both adrenal glands, and, as far as can be ascertained from the records published, only a short time after the administration of prostigmine.

Adler's publication raises a number of important theoretical and clinical problems. It was therefore thought desirable to re-examine the relationship between adrenalectomy and muscle fatigue in acute experiments and to investigate the effect of prostigmine under such conditions.

Methods

The experiments were performed on three dogs and on seventeen cats anaesthetized by intravenous chloralose, 0·08–0·1 g./kg. body weight, or by intraperitoneal 'Nembutal' (sodium pentobarbitone), 30–40 mg./kg. body weight. In a number of experiments lasting up to 12 hr., additional small doses of the anaesthetic had to be given as required. The animals were supine, and the left hind limb was firmly fixed in clamps by means of drills through the femur and the tibia. The contractions of the gastrocnemius-soleus group of muscles were recorded by attaching the tendon to a Sherrington torsion myograph. The muscles were directly stimulated by inserting a needle electrode into their mass and fixing the electrode in position by sutures; the indifferent electrode was placed on the shaved back of the animal.

The stimulating circuit employed a gas discharge triode valve; the usual rate of stimulation was 100/sec. The stimulating circuit was rhythmically interrupted by means of a rotatory circuit breaker so that there were alternate periods of stimulation (0·6–0·8 sec.), and of rest (0·4–0·2 sec.). A control period of stimulation, lasting up to 2 hr., preceded each experiment. In some experiments, before the administration of prostigmine, the leg under observation was denervated to eliminate any possible interfering impulses coming from the central nervous system, in view of the known central action of the drug (Schweitzer & Wright, 1937*a*, *b*).

Both adrenal glands were extirpated, using either the trans- or retro-peritoneal approaches. The completeness of the adrenalectomy was verified in each experiment by post-mortem examination of the animal. Care was taken to avoid loss of blood and, in the post-operational period, cooling of the animals. Arterial blood pressure was recorded from the left common carotid artery. Artificial respiration was used in some of the experiments. All the drugs were dissolved in physiological saline and identical volumes were injected. Acute reductions of the blood volume (blood volume being regarded as 1/11th of the body weight) were produced by rapid bleeding from a femoral artery; the blood was collected in citrate solution and returned to the circulation through the jugular vein by means of a burette (cf. Buttle, Kekwick & Schweitzer, 1940). The experimental results from cats and dogs were identical, and the observations made on both species will be considered together.

Results

Effects of double adrenalectomy

In nine normal animals the contractions of the gastrocnemius-soleus group of muscles, rhythmically tetanized for as long as 2 hr. with stimuli at a frequency of 100/sec., did not show any appreciable degree of fatigue. In two animals the height of muscular contraction was slightly reduced after stimulation

periods of 80 and 95 min. respectively, but in both these cases complete recovery occurred after rest for 5 min.

After extirpation of both adrenal glands, the muscles were stimulated for periods up to 45 min. followed by intervals of rest, occasionally lasting for 60 min.; the observations were continued up to 11 hr. after the adrenalectomy.

In those animals which were maintained in good condition throughout, there was no evidence of any muscular fatigue. Records of the arterial blood pressure taken at intervals during the experiments proved of considerable value in assessing the general condition of the animals. As long as the systemic

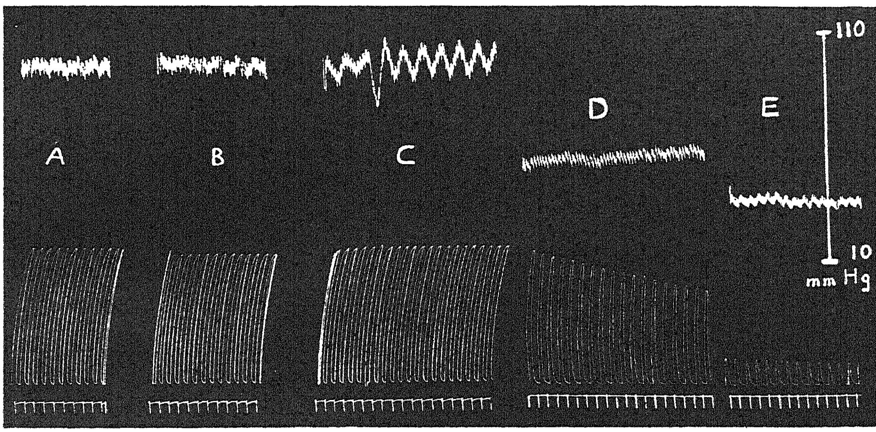

Fig. 1. Records from above downwards are arterial blood pressure, gastrocnemius-soleus contractions, time in 10 sec. Note rapid muscular fatigue with low arterial pressures in sections D and E.

arterial pressure remained above a critical level of 60–70 mm. Hg, signs of muscular fatigue were absent, and the height of the muscular contractions showed no decrease 10 or more hr. after adrenalectomy (sections A–C, Fig. 1).

However, the height of contraction of the muscle group under observation rapidly decreased when the systemic arterial pressure fell and remained, for periods exceeding 3–5 min., below the critical level of 60–70 mm. Hg. In some experiments the muscle in these circumstances became completely unresponsive after tetanic stimulation for only 5–10 min. After rest for 5–30 min., even at low arterial pressures, more or less complete recovery of muscular contraction occurred, but was once more followed by rapid development of fatigue when the stimulation was recommenced. The muscular behaviour always bore a close relationship to the circulatory condition of the animal. Fig. 1 illustrates this intimate dependence of muscular performance on the level of the systemic arterial blood pressure. Sections A, B and C were obtained

2, 4 and 6 hr. respectively, following double adrenalectomy in a cat; the blood pressure fluctuated between 85 and 100 mm. Hg. There was no sign of fatigue after tetanization of the muscle group for 10 min. in each section of the tracing. Sections D and E were recorded 7 and $7\frac{1}{2}$ hr., respectively, after extirpation of both adrenal glands. In both instances fatigue set in rapidly; D shows the response of the muscle after a period of stimulation lasting 3 min., and section E was similarly recorded 2 min. after the onset of stimulation. As will be seen from the records, the arterial blood pressure, during the period of observation recorded in section D, varied between 50 and 60 mm. Hg, while the pressure during period E was approximately 40 mm. Hg. These experiments show that, following double adrenalectomy in acute experiments on cats and dogs, rapid muscular fatigue, on direct tetanizing stimulation, does not set in as long as the arterial blood pressure is maintained at levels above 60–70 mm. Hg. Once, however, the circulatory condition deteriorates and the blood pressure falls below this level, then fatigue develops rapidly to the point of complete unresponsiveness. Indeed, it appears to be very doubtful whether the development of rapid muscular fatigue in these acute experiments is at all causally related to adrenal deficiency, since sham operations, leading to a fall in arterial blood pressure comparable to that occasionally observed following double adrenalectomy, produced identical alterations in muscular performance. This point is borne out more fully in experiments described below, in which the arterial pressure has been acutely diminished by various means.

Effect of arterial hypotension on muscular contraction

Effect of intravenous injection of histamine. Histamine was injected intravenously into normal anaesthetized cats and dogs in doses of 0·1–1·0 mg./kg. body weight. Artificial respiration was used to exclude respiratory embarrassment in some experiments where large doses of the drug were given. The results of these experiments are in full agreement with the previous conclusions. Rapid fatigue in the rhythmically tetanized gastrocnemius-soleus group of muscles developed whenever the arterial blood pressure decreased to levels below 60–70 mm. Hg and was maintained at these low levels for at least 3–5 min. When the effect of the histamine injection wore off, and the blood pressure gradually returned to normal, the muscle contractions progressively increased in amplitude and eventually returned to their pre-injection level. Fig. 2 is representative of this series of experiments. At the arrow in section A, 0·5 mg. of histamine/kg. body weight was intravenously injected into a cat. There was a very rapid diminution of the contraction height of the muscles to approximately 20 % of the pre-injection level as the arterial blood pressure fell from 140 to approximately 60 mm. Hg. Sections B, C and D were obtained at 10, 5 and 5 min. intervals, respectively, after the injection of histamine. It is clearly seen that the return of the amplitude of muscular contraction towards

the pre-injection level coincided with the restoration of the arterial blood pressure to normal, although muscular recovery at the end of section D was not yet complete. In some instances, usually depending on the dose of histamine injected, the restoration of the performance of the muscle to its previous level took as long as 45 min. There is no reason to assume that the effect of histamine in depressing the amplitude of contractions of rhythmically tetanized muscle is attributable to any mechanism other than the profound alteration in blood flow through the exercised muscle during the period of arterial hypotension.

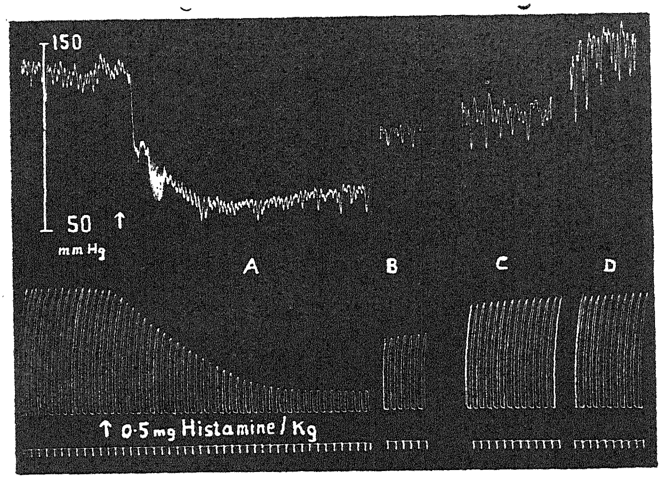

Fig. 2. Records as in Fig. 1. At arrow in A intravenous injection of 0·5 mg. histamine/kg. body weight. Rapid muscular fatigue with fall in blood pressure. B, C and D recorded at 10, 5 and 5 min. intervals, respectively, after histamine injection. Recovery of muscular contractions with return of blood pressure to normal.

Effect of acute haemorrhage and blood transfusion. In this series of experiments the methods employed in bleeding and in transfusion of citrated blood were essentially the same as those described in a previous publication (Buttle *et al.*, 1940). The severity of the haemorrhage varied from 15 to 35 % of the calculated blood volume. In every instance, a fall of arterial blood pressure to values below 60–70 mm. Hg, and maintenance at that low level for some minutes, produced a progressive deterioration of muscular performance. When the blood pressure was not restored to healthier levels, either spontaneously or by means of blood transfusion, the muscle became more or less rapidly unresponsive to direct tetanizing stimulation. In Fig. 3, *H* indicates the period of acute haemorrhage (25 % of the calculated original blood volume) in a normal anaesthetized dog; the citrated blood was transfused during period *T*. The haemorrhage reduced the blood pressure from 130 to approximately

50 mm. Hg and the progressive weakening of the contractions of the stimulated muscle group is clearly seen; the blood pressure returned to 140 mm. Hg after the transfusion of the animal's own citrated blood, and the performance of the muscle quickly returned to the pre-haemorrhage level. It will be seen from this tracing that, once the hypotension was well established, the deterioration of the power of contraction in the muscle was progressive, although during part of period T a slight recovery of the blood pressure to approximately 70 mm. Hg developed; the process of deterioration was finally halted and the muscle fully recovered within 3 min. of the return of the arterial pressure to its normal level.

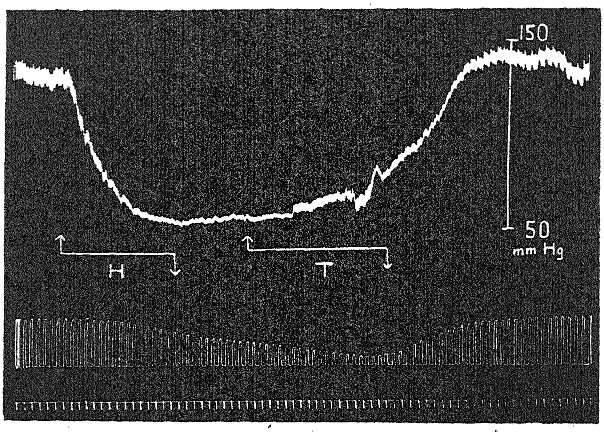

Fig. 3. Records as in Fig. 1. During period H haemorrhage of 25 % of original blood volume, during T transfusion of the citrated blood. Note development of fatigue during period of hypotension and subsequent recovery after the transfusion.

Effect of prostigmine on muscular contractions of adrenalectomized cats and dogs

The effect of prostigmine on normal and on denervated muscle during direct and indirect stimulation has been fully studied by Wilson & Wright (1936), Rosenblueth, Lindsley & Morison (1936) and Schweitzer & Wright (1937 *a*, *b*). As prostigmine, in large doses, has a marked depressant action on respiration (Schweitzer & Wright, 1938) artificial respiration was started before the intravenous injection of the drug into adrenalectomized animals. Furthermore, owing to the potent anti-cholinesterase effect of prostigmine, atropine was given in a number of experiments in doses adequate to prevent serious cardiac embarrassment. Unfortunately, the exact dose of prostigmine which, in Adler's experiments, was capable of restoring to normal the responses of the fatigued muscle in the adrenalectomized dog is not stated, nor is there any record of such precautions as those taken in the present series of experiments.

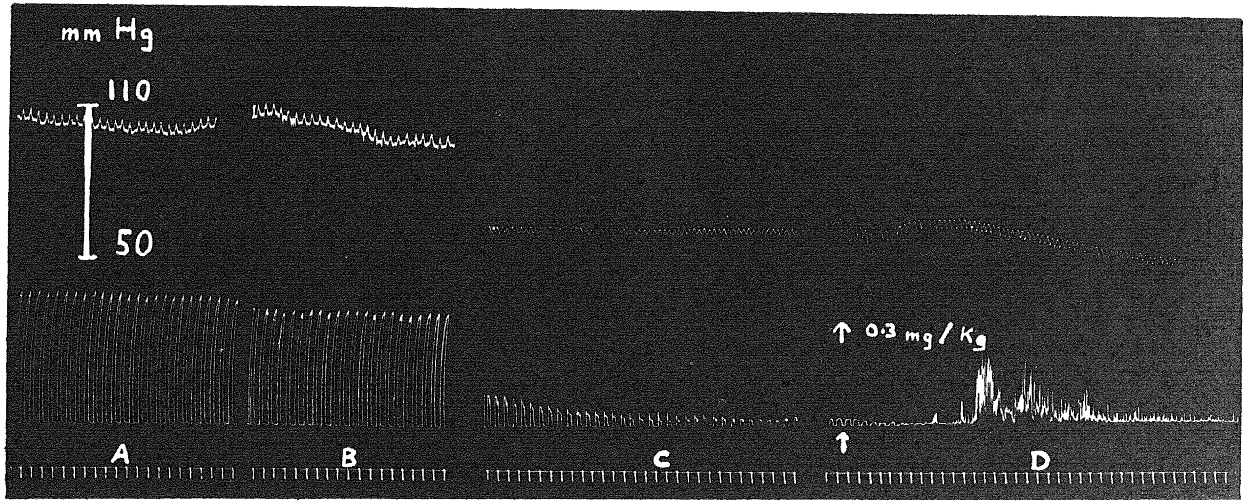

Fig. 4. Records as in Fig. 1. Rapid development of fatigue in C with low arterial pressure. At arrow in D, 0·3 mg./kg. body weight of prostigmine injected intravenously. Muscle becomes unresponsive to tetanizing stimulation; violent fasciculation.

Prostigmine was therefore injected intravenously in doses of 0·1–1·0 mg./kg. body weight in unatropinized and in atropinized adrenalectomized cats and dogs with marked arterial hypotension. The muscles of these animals had shown rapid development of fatigue on tetanic stimulation. In only one out of eight experiments of this type was there a mild 'anti-fatigue' effect after a prostigmine dose of 0·1 mg./kg. body weight. In all other experiments, muscle, which still responded, although to a diminished degree, to direct tetanizing stimulation, rapidly became completely paralysed after the administration of prostigmine. In Fig. 4, *A* shows the response of the muscle before, and *B* 4 hr. after, double adrenalectomy in a cat; in section B the blood pressure fluctuated between 110 and 90 mm. Hg. Section C was recorded $6\frac{1}{2}$ hr. after the removal of the adrenals; the arterial pressure had decreased to 60 mm. Hg and there was rapid development of fatigue; at the arrow in section D 0·3 mg./kg. body weight of prostigmine was intravenously injected after atropinization and commencement of artificial respiration; the muscle rapidly became unresponsive to the tetanizing stimulation, and the record shows intense muscular fasciculation. It has been shown in our previous studies (Schweitzer & Wright, 1937*a, b*) that prostigmine tends to produce more or less violent fasciculation in skeletal muscle. Extensive muscular fasciculation was again observed in our present series of experiments. This point is of some interest in the light of recent clinical studies by Harvey & Lilienthal (1941), on patients suffering from myasthenia gravis, and in view of the effect of prostigmine, given intra-arterially, in normal subjects (Harvey, Lilienthal & Talbot, 1941). This matter will be discussed later.

Discussion

A survey of the available literature on the average survival time of cats and dogs after double adrenalectomy in one-stage or two-stage operations has not revealed much evidence that acute signs of adrenal cortex deficiency are detectable within a few hours of the the removal of the second gland. Trendelenburg (1914) observed normal arterial pressures in cats following one- or two-stage removal of the adrenals for at least 12 hr. after the conclusion of the operations. Grollman (1936, p. 156) states that the average survival time of untreated cats following one-stage double adrenalectomy is 6–7 days. The average survival time of dogs operated upon under ether anaesthesia was up to 21 hr. and, if operated upon under spinal anaesthesia, more than 97 hr. (one-stage procedure—Firor & Grollman, 1933). Rogoff & Dominguez (1927), in observations on non-anaesthetized dogs operated upon in two stages, did not observe any significant changes in the systemic arterial blood pressure for 6 days following the adrenalectomy. Indeed, the situation with regard to the occurrence of early death following removal of the adrenal glands is admirably summed up in the statement of Rogoff & Stewart (1926)—'...when dogs die

in two or three days or less something else than adrenal insufficiency has contributed to shorten life, especially the direct consequences of the surgical procedure.'

The present experiments show clearly that tetanization of muscle in cats and dogs, adrenalectomized in one stage under chloralose or nembutal anaesthesia, does not produce fatigue as long as the circulatory condition of the animals is satisfactory. However, once the systemic arterial blood pressure falls below a certain critical level of 60–70 mm. Hg, and stays at this low value for some minutes, muscular fatigue, under our experimental conditions, rapidly develops. Whether this arterial hypotension is produced by surgical manipulations, such as the acute removal of both adrenal glands, by acute haemorrhage or by intravenous injection of adequate doses of histamine appears to be immaterial for the production of a state of adynamia. Both the experiments in which haemorrhage was followed by return of the lost blood, and the histamine observations, show beyond doubt that this state of muscular fatigue, even if severe, is reversible when the systemic blood pressure is restored to normal. We are, therefore, inclined to regard the onset of muscular fatigue a few hours after removal of both adrenal glands in some cats and dogs as a consequence of the anaesthetic and surgical trauma, leading to circulatory collapse and alterations in the blood flow through the muscle, rather than as a specific result of adrenal deficiency.

In the present experiments, intravenous injection of prostigmine in adrenalectomized animals with low arterial pressures and deteriorating muscular performance was unable to counteract or materially improve the situation. On the contrary, and in agreement with previous investigations (Schweitzer & Wright, 1937*a*,*b*), large doses of prostigmine quickly abolished whatever tetanic muscular contractions were still being observed before the injection of the drug, and produced fasciculation.

Harvey & Lilienthal (1941) and Harvey *et al.* (1941) have convincingly shown that the response of the myasthenic muscle in man to intra-arterially injected prostigmine is quite specific and affords an important differential diagnostic sign. While, in the normal subject, prostigmine injected intra-arterially produces a profound local paresis, presumably due to the local accumulation of paralysing concentrations of normally liberated acetyl choline, in the myasthenic subject it produces a dramatic improvement of muscular power. Furthermore, prostigmine is likely to produce very violent muscular fasciculation in the normal subject, while the myasthenic patient hardly ever shows fasciculation in response to the drug. It will be recalled that, in every instance, prostigmine produced violent fasciculation in our adrenalectomized animals.

These experiments provide no evidence to doubt the specificity of the prostigmine effect in true myasthenia gravis. Prostigmine relieves the symp-

toms of muscular weakness in true myasthenia gravis. It does not, in acute experiments, relieve the adynamia of the adrenalectomized cat or dog which is due to the fall in arterial blood pressure produced by the trauma of the operation; on the contrary, prostigmine aggravates the condition. Our experiments give no evidence to support the statement of Adler that adrenalectomy reproduces the characteristic muscular weakness of myasthenia gravis.

Summary

1. The effect of double adrenalectomy on the amplitude of contractions of the rhythmically tetanized gastrocnemius-soleus group of muscles was studied by means of acute experiments on cats and dogs under chloralose or nembutal anaesthesia.

2. Within the time limit of an acute experiment (12 hr.), adrenalectomy did not lead to adynamia of directly stimulated skeletal muscle so long as the arterial blood pressure was within normal limits. However, if the blood pressure fell to a critical level of 60–70 mm. Hg or less, as a result of operational trauma, then fatigue rapidly developed. There was no evidence in our experiments to show that double adrenalectomy reproduces experimentally the syndrome of true myasthenia gravis.

3. Rapid development of fatigue in the tetanized muscle group, comparable to that observed in certain adrenalectomized animals, followed the production of arterial hypotension by acute haemorrhage or by intravenous injection of histamine. These effects on muscle contractions were reversible with the return of the blood pressure to normal levels, e.g. following blood transfusion.

4. Muscular fatigue, developing in certain acute experiments after double adrenalectomy, could not be relieved by intravenous administration of prostigmine.

While this paper was being prepared for the press, our attention was drawn to a paper by Florey, Szent-Györgyi & Florey (1929). These workers reach conclusions similar to ours.

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THE REGIONAL DISTRIBUTION OF SWEATING

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Kuno's monograph (1934) contains a review of studies which have been made of the variations in the rate of sweating on different parts of the body. The data available refer only to a number of small areas of a few sq.cm. scattered over the surface of the body. A complete analysis of the contribution made by separate anatomical regions such as the arms, legs, trunk, head and neck, to the total sweat loss does not appear to have been made. In addition, there is a lack of data on the changes in the rate of sweating in such regions, when, for some reason, the rate as a whole increases. In this paper an attempt to achieve a complete partition of sweat loss according to such anatomical regions is described.

Methods

Observations were made on three naked male subjects, P., McK. and W., aged respectively 36, 31 and 34 years. These men were being acclimatized to a tropical environment by repeated exposures in a hot room to an atmosphere of dry bulb 100° F., wet bulb 93° F., giving an 'effective' temperature (Houghten, Teague & Miller, 1926) of 94° F.

Every 20 min. during the first 2 hr. of each exposure the men performed a set amount of work. The work consisted of stepping on and off a stool, 1 ft. high, for 5 min., and the grade of work was altered by increasing the rate of stepping on and off. During the first exposure, the first three bouts of work were at such a rate that the men stepped on and off the stool twelve times per min. and, for the last two, twenty-four times per min. During the next four or five exposures the first bout of work only was at the slower rate. In the cases of W. and McK. the duration of the work period was lengthened to 7½ min. in the last three exposures.

These rates of work were so designed as to allow the rectal temperature of each subject to rise to 102° F. or slightly over.

Observations on sweating were made in the 50–60 min. following the last work period, at a time when the subject had a maximum rectal temperature and was sweating profusely.

Sweat was collected from a number of small areas in each anatomical region by the following technique. The area selected was dried for 10 sec., and then a brass ring 7 cm. in diameter and 2 cm. deep was held lightly pressed down on the area. The ring carried a well-fitting lid, to minimize evaporation from the sweat accumulating within the ring. The ring prevented sweat trickling away from the area or reaching it from adjacent areas. After 2 min. the lid was removed, and, during the next ½ min., the sweat within the ring was mopped up by means of a previously dried and weighed cotton pledget. The increase in weight of the pledget was then determined. Thus sweat, accumulating in 2½ min. within the ring from an area corresponding to a plane surface of 38·5 sq.cm., was collected. The actual skin surface under the ring is of course not a flat

surface. By means of the ring, observations were made on thirty such areas scattered over the various anatomical regions of the body. The area on the scalp was dried for a longer time, about 30 to 40 sec., as in one subject the hair was rather damp. As much hair as possible was pushed away from the test area, before applying the ring.

The men were dried with a towel and weighed before and after each bout of work and also at the end of each experiment. During the period of 50–60 min. when sweat was being collected the subjects were weighed twice to keep a check on the constancy of rate of sweating. As, occasionally, the rate of sweating was found to decrease in this period, the 'effective' temperature was raised 1 or 2° F. in the last 20–30 min. of the experiment. This helped to maintain the sweat rate constant.

The sequence of areas, from which the sweat was collected, was so arranged that the sweat was obtained from three successive groups each of ten areas. Each group included areas from every region, so that the total rate of sweating of each group reflected the rate of sweating of the body as a whole. In choosing comparable groups of areas reliance was placed on figures obtained for the rates of sweating of the various areas in exploratory experiments on subject P. Only experiments were used in which the total sweat rate remained constant.

The surface area of the various regions of the three subjects was found by taking the measurements and using the formulae suggested by du Bois & du Bois (1915).

Results

The number of areas from which sweat was collected in each anatomical region is shown in Table 1. The table also gives the percentage of the total body surface occupied by each anatomical region, these percentages representing the average for the three subjects P., McK. and W. (Table 2). These values are quite similar to those of an individual (surface area 1·8 sq.m.) studied by du Bois & du Bois (1915).

Table 1. The percentage of the surface area in each region from which sweat was collected

Anatomical region	Regional area sq.cm.	Each region as % of total skin surface	No. of test areas in region	Total test area per region sq.cm.	Total test area as % of regional area
Head	1087	6·6	4	154·0	14·1
Trunk	6476	39·3	10	385·0	5·9
Thighs	2310	14·0	4	154·0	6·7
Legs	2153	13·1	3	115·5	5·4
Feet	1155	7·0	2	77·0	6·7
Arms and forearms	2234	13·6	5	192·5	8·6
Hands	1035	6·3	2	77·0	7·4
Totals	16450	99·9	30	1155·0	—

The fact that in some regions sweat is collected from relatively more of the surface than in other regions is to a large extent unavoidable. In some cases the accessible surfaces comprise a larger proportion of the region, as for example the head, hands, arms, and forearms. In other cases the contrary is true, and the convenient areas for applying the ring and collecting the sweat are limited. This is so over the legs. More areas could have been used on the trunk and thighs, but this gain would have been offset by the longer time required to carry out the experiments. In any case the areas chosen seemed on the whole adequately representative of these regions.

Having determined the rate of sweating of each particular test area in each anatomical region, the average rate of sweating of each anatomical

TABLE 2. Surface area (sq.cm.) of body regions and total surface estimated according to methods of du Bois & du Bois (1915)

	Subject P.		Subject McK.		Subject W.	
Body region	Area sq.cm.	% of total area	Area sq.cm.	% of total area	Area sq.cm.	% of total area
Head	1,192	6·6	1,009	6·3	1,060	7·0
Trunk	7,166	39·2	6,337	39·6	5,925	39·3
Thighs	2,694	14·7	2,125	13·3	2,110	14·0
Legs	2,314	12·6	2,231	14·0	1,914	12·7
Feet	1,243	6·8	1,079	6·8	1,142	7·6
Arms and forearms	2,544	13·9	2,268	14·2	1,889	12·5
Hands	1,143	6·2	929	5·8	1,035	6·9
Totals	18,296	100·0	15,978	100·0	15,075	100·0
Surface area (sq.cm.) from formula of du Bois	18,113	—	16,670	—	15,169	—

The total area of the body surface was calculated from the du Bois formula:

$$A = W^{0\cdot425} \times H^{0\cdot725} \times 71\cdot84,$$

where A is surface area in sq.cm., W is body weight in kg. and H is standing height in cm.

The body measurements of subjects P., McK. and W. were, for standing height, 177·8, 166·1 and 158·4 cm. respectively, and for weight, 65·0, 60·0 and 55·0 kg. respectively.

region per 100 sq.cm. surface was calculated (Table 3). The value per sq.cm. surface was multiplied by the surface area of the region to give the total rate of sweating of the anatomical region. This calculation of the regional rate of sweating involves the assumption that each anatomical region behaves uniformly all over its surface with regard to sweating and that the chosen test areas are truly representative of the region. This assumption is considered below in relation to the results obtained.

Local variations in sweating

Table 3 shows the rate of sweating (mg./min./100 sq.cm.) in the subdivisions of each anatomical region, together with the average rate for each region as a whole. The marked variation in the different areas is apparent—a fact emphasized by Kuno (1934). The low sweating of the palm and the sole are due to the fact that the sweat glands of these localities are far more sensitive to mental stimuli than to thermal. Consistent differences between particular areas as shown in Table 3 are to a large extent in agreement with those described by Ikeuchi & Kuno (quoted by Kuno, 1934), who studied three Japanese males and three Japanese females. With nearly the whole of the following statement, representing Kuno's main conclusions, one can agree, though with some reservation regarding the rate of sweating on the cheek and on the extensor surface of the forearm. The axilla proper was not studied in the present investigation. Kuno's statement (1934) is as follows:

> ...we may classify the body surface into the following parts according to the profuseness of sweating: (1) the forehead, the neck, some larger areas of the anterior and posterior surfaces of the trunk, the lumbar region, the dorsal region of the hand and the adjacent part of the forearm are the parts which sweat most. (2) The cheek, the lateral surface of the trunk and the greater part of the extremities sweat remarkably less than the former. (3) The internal femoral region and the axilla sweat still less. (4) The palm and the sole are the parts which sweat least.

TABLE 3. Local sweating (mg./min./100 sq.cm.)

	Subject P.			Subject McK.			Subject W.		
	Day 4	Day 6	Day 8	Day 4	Day 7	Day 11	Day 3	Day 6	Day 9
Head:									
Scalp	—	13	13	28	33	60	7	11	0
Forehead	46	209	208	30	47	50	137	130	199
Cheek	—	—	27	110	144	184	84	71	49
Neck	73	82	85	32	110	187	74	79	91
Average for head	**59·5**	**101·3**	**83·2**	**50·0**	**83·5**	**120·2**	**75·5**	**72·7**	**84·7**
Trunk:									
Upper chest	119	105	111	113	120	198	140	173	143
Lower chest	—	—	102	117	122	193	114	189	100
Upper abdomen	81	93	102	115	60	109	96	157	134
Lower Abdomen	70	80	—	87	49	148	59	113	80
Suprascapular region	62	92	95	70	81	76	93	101	120
Scapular region	49	65	62	60	29	86	91	118	138
Infrascapular region	—	88	—	48	—	93	67	31	120
Lumbar region	14	79	80	35	—	71	53	54	104
Upper axillary region	—	—	—	103	33	125	85	126	127
Lower axillary region	—	—	—	82	36	94	70	40	146
Average for trunk	**66·0**	**86·0**	**92·2**	**83·0**	**66·2**	**119·3**	**86·8**	**110·2**	**121·2**
Thighs									
Medial surface	20	34	37	14	—	31	23	23	49
Lateral surface	47	86	79	38	35	44	99	118	96
Posterior surface	22	40	62	27	34	39	68	76	96
Buttock	48	32	57	14	12	24	54	21	62
Average for thighs	**34·3**	**48·0**	**58·7**	**23·3**	**27·0**	**34·5**	**61·0**	**59·5**	**75·7**
Legs:									
Medial surface	38	67	44	50	52	61	74	86	100
Lateral surface	—	65	56	51	35	44	76	94	110
Posterior surface	—	—	—	27	26	25	73	—	130
Average for legs	**38·0**	**66·0**	**50·0**	**42·6**	**37·6**	**43·3**	**74·3**	**90·0**	**113·3**
Feet:									
Sole	9	13	22	19	8	10	12	11	16
Dorsum	3	61	80	40	25	21	28	23	21
Average for feet	**9·0**	**37·0**	**51·0**	**29·5**	**16·5**	**15·5**	**20·0**	**17·0**	**18·5**
Arms and forearms:									
Shoulder	34	55	43	47	25	23	95	40	—
Deltoid region	42	36	40	52	28	45	43	34	—
Medial surface upper arm	—	—	—	28	21	34	43	29	16
Extensor surface forearm	28	—	23	25	42	116	46	82	46
Flexor surface forearm	—	—	—	56	86	99	75	52	61
Average for forelimb	**34·6**	**45·5**	**35·3**	**41·6**	**40·4**	**63·4**	**60·4**	**47·4**	**41·0**
Hands:									
Back of hand	25	22	22	15	21	33	59	26	43
Palm	3	5	9	0	7	10	7	0	7
Average for hands	**14·0**	**13·5**	**15·5**	**7·5**	**14·0**	**21·5**	**33·0**	**13·0**	**25·0**

The following additional points may be made on the basis of the figures set out in Table 3:

(1) There is no consistent difference between the rate of sweating on the medial and lateral surfaces of the leg and the arm.

(2) The individual differences in the amount of moisture one can collect from the scalp is marked. Subject McK., not bald, sweated apparently at about the same rate on the scalp as on the forehead. In the other subjects the scalp was usually nearly dry, whereas the forehead sweated very profusely.

(3) There is some suggestion in the figures of Table 3 that sweating on the back is less than on the front of the trunk, if roughly the same anatomical levels are considered. There seems also a tendency for the rate of sweating to diminish the more caudal the area on the trunk.

Regional distribution of sweating

In Table 4 is given the rate of sweating of each of the seven anatomical regions of the body surface. The rate of sweating in g./hr. of the individual regions was calculated from the average rate of sweating of the test areas investigated in the region (Table 3) and the estimated surface area of the region (Table 2). The 'calculated total' is obtained by adding together the rates for the individual regions, and the result may be compared with the actual loss measured directly by loss of body weight. The change in weight due to water loss in respiration and to the excess of oxygen retained over carbon dioxide eliminated was neglected.

The correspondence between the calculated and the observed loss may be regarded as not unsatisfactory. The agreement obtained depends no doubt to some extent on the mutual cancellation of errors in the various regions. It suggests, however, that the method of calculation does not involve gross errors, particularly in the assumptions that the area of collection corresponds to a plane area under the ring and that the average of the areas used may be taken to represent the regions as a whole. There is one factor inherent in the method which will counteract any tendency to overestimation of the sweat rate which may result from the assumptions made. Not all the sweat produced during the 2 min. periods can actually be collected. During the $\frac{1}{2}$ min. when the area is dried, evaporation must be fairly high owing, not only to the exposure of the sweating area to an atmosphere of suddenly reduced vapour pressure, but also to the air movement created as the area is being dried. It is possible that only half the sweat actually produced in the $\frac{1}{2}$ min. is picked up. Assuming that the pledget collects the sweat produced in the preceding 2 min. without loss, then only $2\frac{1}{4}$ min. may be represented in the $2\frac{1}{2}$ min. collection—a loss of 10%. This loss, on the whole, may be fairly constant though it will vary with the skin temperature and air conditions prevailing. A fairly constant error in the opposite direction arises from the tendency to take rather more sweat off the curved skin surface under the ring than allowed for by the area in the calculation.

Conclusions

In spite of the limitations of both method and calculation it seems possible to draw certain general conclusions from the figures available.

(*a*) *Partition of sweating.* Examination of Table 4 reveals that in all three subjects, as a rough generalization, about 50% of the sweating comes from the trunk, about 25% is derived from the lower limbs, and the remaining

Table 4. Regional distribution of sweating.

Subject P.

	Day 4		Day 6		Day 8	
	g./hr.	% of total sweat	g./hr.	% of total sweat	g./hr.	% of total sweat
Head	43	8·2	73	10·1	60	8·3
Trunk	284	54·4	370	51·4	396	54·8
Thighs	55	10·5	78	10·8	95	13·1
Legs	53	10·1	92	12·8	69	9·5
Feet	*25	4·8	28	3·9	38	5·3
Arms and forearms	53	10·1	70	9·7	54	7·5
Hands	10	1·9	9	1·3	11	1·5
Calculated total	523	100·0	720	100·0	723	100·0
Observed total	524	—	664	—	680	—
Percentage difference:						
Calculated			108/100		106/100	
Observed	0	—	+8	—	+6	—

Subject McK.

	Day 4		Day 7		Day 11	
	g./hr.	% of total sweat	g./hr.	% of total sweat	g./hr.	% of total sweat
Head	30	5·9	51	11·1	73	9·9
Trunk	316	61·6	252	54·6	454	61·5
Thighs	30	5·8	34	7·5	44	6·0
Legs	57	11·1	50	10·8	58	7·9
Feet	19	3·7	11	2·4	10	1·4
Arms and forearms	57	11·1	55	11·9	86	11·7
Hands	4	0·8	8	1·7	12	1·6
Calculated total	513	100·0	461	100·0	737	100·0
Observed total	485	—	470	—	725	—
Percentage difference:						
Calculated	106/100		98/100		102/100	
Observed	+6	—	−2	—	+2	—

Subject W.

	Day 3		Day 6		Day 9	
	g./hr.	% of total sweat	g./hr.	% of total sweat	g./hr.	% of total sweat
Head	48	7·7	46	6·7	54	6·9
Trunk	308	49·6	391	56·7	430	54·8
Thighs	77	12·4	75	10·9	96	12·2
Legs	85	13·6	103	14·9	130	16·5
Feet	14	2·3	12	1·7	13	1·7
Arms and forearms	68	11·0	54	7·9	46	5·9
Hands	21	3·4	8	1·2	16	2·0
Calculated total	621	100·0	689	100·0	785	100·0
Observed total	660	—	740	—	800	—
Percentage difference:						
Calculated	94/100		92/100		98/100	
Observed	−6	—	−8	—	−2	—

* In absence of data this value is assumed to be not less than that of day 6.

25% comes from the head and the upper limbs. The lower limbs (thighs, legs and feet) contribute more than the upper (arms, forearms and hands). It is an interesting fact that the legs contribute as much as, and often more than, the thighs. Differences between individuals are quite marked as shown in the figures given in Table 4. Subject McK. produces a markedly smaller proportion of sweat on the thighs and perhaps rather more on the trunk than do the other subjects. Subject P. produces a bigger proportion on the feet. The proportions derived from head, legs, forelimbs and hands are roughly similar in the three subjects.

(*b*) *Changes in rate of sweating.* In the third of each series of three experiments on each subject a rate of sweating greater than that of the preceding days was found, due in large part to the process of acclimatization. During this process certain regional changes appear to be consistently connected with the total increase in sweating which occurs. The loss from the trunk and the thigh appears to be invariably increased and is responsible for the bulk of the increase, though not to the same extent in the three subjects. The changes in sweating on hands and feet are rather irregular though small. The sweating on the legs generally increased, but on the arm and forearm the results are irregular.

(*c*) *Relative intensity of sweating.* It is evident that the sweat rate per unit area is markedly different in different regions. This can be shown by a comparison between the percentage which each region contributes to the total sweating and the percentage of the total surface area of the body represented by each region. The ratio between these figures is a measure of the 'relative intensity' and is given in Table 5. An average has been calculated for all three

TABLE 5. Relative intensity of sweating over various regions of body surface

$$\text{Relative intensity} = \frac{\text{Proportion of total sweating contributed by region}}{\text{Proportion of total surface represented by region}}.$$

	Subject P.				Subject McK.				Subject W.			
Region	Day 4	Day 6	Day 8	Av.	Day 4	Day 7	Day 11	Av.	Day 3	Day 6	Day 9	Av.
Head	1·3	1·5	1·3	1·4	0·9	1·8	1·6	1·4	1·1	1·0	1·0	1·0
Trunk	1·4	1·3	1·4	1·4	1·6	1·4	1·6	1·5	1·3	1·4	1·4	1·3
Thighs	0·7	0·7	0·9	0·8	0·4	0·6	0·5	0·5	0·9	0·8	0·9	0·8
Legs	0·8	1·0	0·8	0·9	0·8	0·8	0·6	0·7	1·1	1·1	1·3	1·2
Feet	0·7	0·6	0·7	0·7	0·5	0·4	0·2	0·4	0·3	0·2	0·2	0·2
Arms and forearms	0·7	0·7	0·5	0·6	0·8	0·8	0·8	0·8	0·9	0·6	0·5	0·7
Hands	0·3	0·2	0·2	0·2	0·1	0·3	0·3	0·2	0·5	0·2	0·3	0·3

days in each case since changes in sweat rate do not, for our present purpose, make any great difference to this ratio. Definite individual differences are revealed, but in general it appears that the average intensity of sweating on the trunk is greater than elsewhere. In all the other regions, with the exception of the head (and of the legs in subject W.), the contribution to the total sweating is less than one would expect from the area involved. The 'intensity'

of sweating on the hands and feet is, in general, notably less than that of other regions, being one-fourth to one-fifth of that on the trunk and one-half to one-third of that on the other regions.

Discussion

The results presented above make it clear that variations in sweating between different regions of the body may be quite considerable, and that between particular small areas of the skin surface the differences may be very large indeed. How far these differences are related to the number, the size or the activity of the sweat glands in each area cannot be definitely answered. However, by the use of some such method as that described in this paper, especially on restricted areas, it may be possible to reveal whether or not a close correlation exists between the total number of sweat glands and their physiological activity as measured by sweat production. If such a correlation were found, it would imply that all glands in a given area are to some extent active even over a short period of time. An increase in sweating in a given area would then depend not on the employment of hitherto unused glands but on a more intense average activity of all the glands. It is equally possible, however, that in some cases at least, as for example in acclimatization to heat, the increase in sweating may ultimately be dependent on a morphological hypertrophy of the sweat glands.

The present results, in the absence of data as to the number of sweat glands in each area, do not permit any definite conclusion as to the functional or anatomical factors underlying variations in intensity of sweating. The fact that in some regions the rate of sweating increases relatively more than in others, when the overall rate of sweating increases, does not favour particularly either of the alternative explanations. It may be that in some areas, e.g. the trunk, there is a greater reserve of 'unused' glands, so allowing a greater increase, or it may be that the individual glands are on the average larger, or for some reason, are able to secrete more intensely.

Certain of Kuno's experiments (1934) support the findings of Jurgensen (quoted by Kuno, 1934), that the rate of secretion of a sweat gland varies according to the intensity of stimulation. He believes, therefore, that changes in the amount of sweating may be due partly to the variable activity of each acting sweat gland. In addition, he believes that the number of sweat glands in action may increase, especially as the sweating during a given time increases. The observations of Saito (quoted by Kuno, 1934) on thermal sweating from the back of the hand indicate that individual sweat glands vary in excitability and that on different days in the same locality previously inactive glands may come into action. Kuno's final conclusion is that changes in rate of sweating are due more essentially to the number of active sweat glands. However, it

still remains to be decided how far these findings apply in a quantitative fashion to variations in sweating in the different regions of the body surface and in those regions where the rate of sweating varies.

Summary

1. A method is described for measuring the rate of sweating from small areas of the skin surface.

2. The rate of sweating from thirty small areas has been measured by this method.

3. By using the average rate of sweating and by measuring the surface area, the total rate of sweating has been approximately partitioned between the head, trunk, thighs, legs, feet, arms and forearms.

4. The possible factors responsible for variations in intensity of sweating in different regions and for changes in sweating in the same regions are discussed.

Dr B. S. Platt and Mr J. C. D. Hutchinson participated in the development of the method for sweat collection described in the paper. I am indebted to Dr E. Arnold Carmichael for advice and encouragement and to Dr W. S. S. Ladell and Mr T. Glenister for help during the investigations. The work was made possible by the support of the Medical Research Council.

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THE DIGESTIBILITY OF THE PHYTATE-P OF OATMEAL IN ADULT MAN

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(*Received* 9 *October* 1944)

The opinion is widely held that oatmeal may have a detrimental effect upon Ca absorption because of its high content of phytate-P. The only investigation undertaken specifically to study this effect in man is that of Burton (1929–30), who compared absorptions and retentions of Ca and P in children and adults receiving wheat and oatmeal diets. This worker found better retentions of Ca when wheat diets were taken. The children were irradiated with ultra-violet light, and in four cases out of six the Ca absorptions and retentions were several times the normal values established by Duckworth & Warnock (1942). Further, the Ca intake was not equalized, so that in all subjects but one the Ca intake was greater on the wheat than on the oatmeal diet. Steggerda & Mitchell (1939), who incidentally used a basal diet rich in oatmeal to keep the Ca intake low, found Ca absorption and requirement within normal ranges.

Since McCance & Widdowson (1935, 1942) found that, under their experimental conditions, adult man excreted approximately half the ingested phytate-P of wheaten flour and that the phytates seriously interfered with the absorption of dietary calcium, it appeared desirable to investigate the utilization of phytate-P in oatmeal at various levels of Ca intake. At the same time it was thought desirable to follow the balances of Ca and Fe.

METHODS

The general pattern of the diet was made to conform, as far as possible under the conditions of the experiment, with that found by Cathcart, Murray & Beveridge (1940) to prevail in the Scottish Highlands. To keep the Ca content of the diet low, cheese was eliminated and milk intake reduced. Since the oatmeal intake in the Highlands is lower to-day than hitherto, the allowance was increased to bring it closer to that consumed during last century (Hutchison, 1868). This led to a corresponding reduction in flour intake.

To simplify the experimental procedure, to reduce the amount of analysis, and to minimize the variability of factors other than Ca intake, a constant diet was eaten throughout the study. This was made possible by the collaboration of the Low Temperature Research Station and the Torry Research Station, both of the Department of Scientific and Industrial Research, to whom our thanks are due for generous gifts of dehydrated foodstuffs and frozen fish.

The oatmeal was taken in the form of oatcakes baked without the inclusion of wheaten flour but with the usual amount of baking powder added. Additional cereal was supplied in the form of low-extraction white-flour biscuits. The oatcakes and biscuits were made in a single batch before the beginning of the experiment, and the mixing and baking were carried out under supervision to minimize risks of contamination with Fe in the form of rust.

The fish was cleaned, filleted and minced; each piece was examined to ensure the complete removal of bone. The ground mass was well mixed, weighed into a sufficient number of portions to cover the study and stored at -30° C. Each day's requirement was drawn fresh from the store. There was no deterioration in appearance or palatability during the 9 weeks' storage period.

As with the fish, each subject's rations of dehydrated meat, dehydrated cabbage, dehydrated potato, oatcakes and biscuits were weighed and packeted before the experiment began. The dehydrated carrots could not be weighed in advance because when in contact with air for long periods they develop distasteful flavours. Consequently a single batch of dehydrated carrots was divided into twelve tins which were then filled with nitrogen and sealed. Four such tins were used for each experiment, one being opened and analysed at the beginning of each period. Small differences in the Ca, Fe and P intakes from one experiment to another were caused by slight alterations in milk composition from day to day, and also by slight variations in carrot composition from tin to tin, occasioned partly by changes in moisture content. As will be seen from the records these variations were of no importance.

Bulk supplies of margarine and bramble jelly were obtained at the beginning of the experiment and the required amounts weighed daily. Milk was received daily from the same herd, and daily samples of milk were combined and analysed for each period. In the two experiments where supplements of Ca lactate were given, an appropriate volume of a stock solution was added to each subject's milk allowance.

A preliminary study was undertaken to determine the amounts necessary to satisfy individual appetites. All subjects were given the same amount of each constituent except biscuits. Subject D. T. ate no potato. Each individual adjusted the quantity of biscuits eaten to satisfy appetite. The amounts established at this time were maintained throughout the study which was continuous and lasted for 48 days. The intakes of the different dietary constituents are given in Table 1.

TABLE 1. Composition of diet consumed daily

	g.		g.
Dehydrated meat	40	Biscuits: Subject J. D.	80
Dehydrated cabbage	15	,, A. H.	165
Dehydrated carrots	10	,, W. S.	210
Dehydrated potatoes	40	,, D. T.	100
Fish	120		
Oatcakes	165		mg.
Margarine	40	Calcium supplement: Exp. 2	200
Jam	40	Exp. 3	500
Milk	250		

Phytate-P intake derived from oatcakes

	% of total
Subject J. D.	94·8
,, A. H.	90·3
,, W. S.	88·1
,, D. T.	94·0

Breakfast, at 9 a.m., consisted of oatcakes, margarine and jam; lunch, at 1 p.m., of boiled meat, boiled cabbage and boiled potatoes; tea, at 5 p.m. of boiled fish, boiled carrots and boiled potatoes; supper, at 10 p.m., of biscuits and margarine. No effort was made to control strictly the division of biscuits between different meals; each subject chose a satisfactory division of the biscuits, margarine and jam over the four meals and adhered closely to it throughout the experiment. Subject D. T. drank all her milk and ate most of her oatcakes at breakfast. Subjects J. D. and

A. H. drank most of their milk at breakfast time and the remainder at lunch and tea; W. S. ate all his oatcakes at breakfast, but he retained all his milk until evening when it was consumed with the remaining white-flour biscuits at supper.

All cooking was done in the laboratory by one subject (D. T.), each individual's meal being cooked separately in glass vessels. Distilled water was used for cooking and drinking. All cooking liquors were consumed.

Three experiments of four periods each were conducted. The diet was the same throughout the study except that it was supplemented with 200 mg. Ca, as lactate, during Exp. 2 and with 500 mg. during Exp. 3. At the beginning of each experiment there was a preparatory period of 4–9 days, for the subject to become adjusted to the alteration, and no collections of urine or faeces were made at this time. During the succeeding three periods customary balance technique was observed; each period lasted 4 days with the exception of period 3 of Exp. 3 which lasted 3 days. All samples of faeces were mixed with an equal volume of alcohol and 20 ml. toluene and a few ml. formaldehyde added. Carmine was used as a faecal marker to separate periods.

The following methods of analysis were used: all Ca determinations, the method of the Association of Official Agricultural Chemists; urinary P, method of Fiske & Subbarow (1925) on the ash of urine dried and ignited with an excess of Ca acetate; food and faecal P, the method of Richards & Godden (1924) on samples dried and ignited with an excess of Ca acetate; Fe in foods and faeces, the method of McFarlane (1932). The phytate-P in foods and faeces was determined by a modification of the method of McCance & Widdowson (1935); $N/2$ trichloroacetic acid was used instead of $N/2$ HCl for the extraction of phytic acid; the same values of phytate-P were found in $N/2$ CCl_3COOH and $N/2$ and $2N$ HCl extracts of faeces. No evidence of non-extractable Fe phytate was found: the acid-extracted faeces did not yield significant quantities of Na phytate on treatment with $N/2$ NaOH. Instead of ashing the whole of the filtrate from the Fe hydroxide precipitate and determining the P in an aliquot by the method of Briggs, an aliquot of the filtrate from the Fe hydroxide precipitate was ashed and the P determined by the method of Fiske & Subbarow. The inorganic P in the filtrate from the Fe hydroxide precipitate was also determined and deducted from the value obtained by incineration. Although ordinarily small this value increased when the ratio of phytate-P to inorganic P in the food stuff or faeces became small, and caused the phytate-P values to be too high.

The subjects were normal. J. D., a male, age 35 years, weighed 179 lb. A. H., a male, age 19 years, weighed 122 lb. W. S., a male, age 33 years, weighed 133 lb. D. T., a female, age 21 years, weighed 148 lb. Subjects A. H. and W. S. are of an active type while J. D. and D. T. tend to be lethargic.

Results

Although analyses were performed for each period, only average values for each experiment are presented in order to conserve space. When necessary, means have been weighted. The term 'digestibility', when applied to phytate-P or dry matter, refers to the disappearance of these substances from the ingesta during their passage through the intestine irrespective of whether it is due to the subject's own digestive action or that of the intestinal flora. The term 'absorption' refers to the difference between intake and faecal output in the case of mineral constituents.

Digestibility of phytate-P and of dry matter (Table 2). The mean digestibility of phytate-P was 94 % in Exp. 1, where Ca intake fell just short of requirement. In all subjects, except W. S. who showed no alteration and who took his Ca and oatmeal separately, fortification of the diet with Ca in Exps. 2 and 3 was associated with a depression in the digestibility of phytate-P; but even on the highest Ca intake the lowest digestibility found was 66 %.

TABLE 2. Average digestibilities of phytate-P and dietary dry matter

		Subject				
		J. D.	A. H.	W. S.	D. T.	Mean
Daily intake of phytate-P (mg.)*		557	585	600	562	574
% Phytate-P digested:	Exp. 1	94	100	86	94	94
	Exp. 2	89	97	88	94	92
	Exp. 3	75	79	86	66	77
% Dry matter digested:	Exp. 1	95·2	96·5	94·0	95·6	95·4
	Exp. 2	94·5	95·8	95·2	95·9	95·3
	Exp. 3	95·0	95·3	94·9	96·4	95·4

* Variations in carrot phytate-P were insignificant and intakes remained unchanged throughout.

The percentage digestibility of the dry matter varied over a narrow range. The mean values were quite constant.

TABLE 3. Average daily P balances (mg.)

	Subject				
	J. D.	A. H.	W. S.	D. T.	Mean
		Exp. 1			
Intake	1861	2022	2106	1899	1972
% Absorbed	66·8	70·6	54·2	70·0	66·4
Urine	1262	1315	1319	1191	1267
Faeces	618	595	966	569	662
Balance	−19	+112	−179	+139	+43
		Exp. 2			
Intake	1848	2008	2094	1886	1941
% Absorbed	57·4	62·2	66·0	69·5	63·7
Urine	1113	1197	1257	1039	1136
Faeces	788	760	713	576	704
Balance	−53	+51	+124	+271	+101
		Exp. 3			
Intake	1853	2014	2098	1891	1964
% Absorbed	59·0	57·4	59·5	63·5	59·8
Urine	1091	1055	1220	1001	1092
Faeces	759	858	849	691	789
Balance	+3	+101	+29	+199	+81

Absorption of P (Table 3). The average percentages of the intakes voided in the faeces during Exps. 1, 2 and 3 were 34, 36 and 40 respectively. When the unhydrolysed phytate-P is subtracted from the total faecal P these percentages become 32, 34 and 33. This indicates that the slight increases observed arise almost quantitatively from decreased phytic acid hydrolysis and therefore that the P arising from the breakdown of phytic acid is absorbed to the same extent as P of non-phytate origin.

Absorption of Ca *and* Fe. The data for the absorption of Ca and Fe are given in Tables 4 and 5.

DISCUSSION

There are two findings of interest: (1) The phytate-P of oatmeal was almost completely digested by four adult subjects when the Ca intake approached requirement, and the P of the hydrolysed phytic acid was absorbed from the

TABLE 4. Average daily Ca balances (mg.)

	Subject				
	J. D.	A. H.	W. S.	D. T.	Mean
			Exp. 1		
Intake	583	598	614	545	582
% Absorbed	18·5	23·4	−13·4	7·3	10·8
Urine	208	112	200	107	153
Faeces	475	458	696	505	519
Balance	−100	+28	−282	−67	−90
			Exp. 2		
Intake	769	784	792	730	765
% Absorbed	10·3	10·5	27·5	24·2	17·9
Urine	239	101	264	85	170
Faeces	690	702	574	553	628
Balance	−160	−19	−46	+92	−33
			Exp. 3		
Intake	1076	1091	1099	1038	1076
% Absorbed	17·1	8·8	22·4	15·1	15·9
Urine	287	118	346	102	213
Faeces	892	995	853	881	905
Balance	−103	−22	−100	+55	−42

TABLE 5. Average daily Fe absorptions (mg.)

	Subject					
	J. D.	A. H.	W. S.	D. T.	Mean	% absorbed
			Exp. 1			
Intake	18·1	19·9	20·6	17·2	19·0	
Absorbed	0·4	0·5	0·4	0·6	0·5	2·6
			Exp. 2			
Intake	18·2	20·0	20·9	17·3	19·1	
Absorbed	0·4	0·3	0·6	0·8	0·5	2·6
			Exp. 3			
Intake	18·3	20·1	21·0	17·4	19·2	
Absorbed	0·6	0·6	0·7	0·9	0·7	3·6

intestine. (2) Addition of Ca to the diet decreased the digestibility of phytate-P if the supplementary Ca was taken together with the oatmeal (subjects J. D., A. H., D. T.) and not if it was taken separately (subject W. S.).

The first finding is unexpected. McCance & Widdowson (1935) found in adults taking wheaten flour (Hovis bread) as sources of phytic acid that an average of 46 % of the ingested phytic acid was excreted unchanged. No data regarding Ca intakes were given. In our experiments with a phytic acid intake more than 50 % higher than that in the experiments of McCance & Widdowson, only 6, 8 and 23 % were excreted unchanged when the daily Ca intakes were 582, 765 and 1076 mg. It is most unlikely that these great differences between the digestibilities of phytic acid derived from wheaten flour and from oatmeal are caused by difference in the experimental subjects. With the facts available at present we are not in a position to offer an explanation for the observed difference.

SUMMARY

In experiments on four adult subjects consuming a constant diet rich in oatmeal, it has been found that, when Ca intake approached requirement, the phytate-P of oatmeal was almost completely digested and P absorption was normal. Increased Ca intakes depressed to some extent the digestibility of phytate-P.

We wish to acknowledge our indebtedness to A. H., W. S., and D. T. for their co-operation and, in the case of A. H. and D. T., for laboratory assistance.

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TESTING DIPHENYLETHYLAMINE COMPOUNDS FOR ANALGESIC ACTION

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Diphenylethylamine and compounds related to it have some of the pharmacological properties of morphine: they may depress the righting reflex in rats, raise the blood-sugar level in rabbits, and produce nausea, hyperexcitability and pupil dilatation in cats (Dodds, Lawson & Williams, 1943, 1944). If the compounds are to be of clinical value they must produce analgesia. Preliminary clinical trials of four of the compounds showed that two of them (diphenylethylamine and hydroxy-diphenylethylamine) relieved the pain of patients suffering from the pressure effects of secondary deposits in malignant disease. The present paper records an attempt to estimate the analgesic potency of the compounds, but no such action could be demonstrated by the methods adopted.

TOLERANCE OF HEAT PAIN IN MAN

Method

The method of Hardy, Wolff & Goodell (1940) was used. The beam from a kilowatt lamp is concentrated for 3 sec. on a blackened area of the subject's forehead. There is a variable resistance in the circuit and the radiant energy is raised until the subject reports that the sensation of heat has changed into one of pain. The energy output of the lamp at each resistance was determined by voltmeter and ammeter readings. In view of the unsatisfactory results there was no need to calibrate the apparatus in terms of calories falling on the test area. Readings were made at 15 min. intervals.

Two subjects were each given one or two cachets by mouth, after four control readings had been made. The test was continued for 3 hr. after the cachet had been given. The subjects were ignorant of the contents of the cachets, which were either morphine hydrochloride (11 mg.), or diphenylethylamine hydrochloride (200 mg.), or lactose (200 mg.).

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Results

The mean minimum wattage at which pain was felt during the control period was calculated and the subsequent readings expressed as a percentage of this figure. The maximum increases are recorded in Table 1, which shows that neither 22 mg. of morphine hydrochloride nor 200 mg. of diphenylethylamine hydrochloride given by mouth produce any significant rise in pain threshold.

Table 1. Pain threshold measurements after taking cachets by mouth

Contents of cachet		Maximum threshold recorded as percentage of control reading: Subject 1	Subject 2
Lactose:	200 mg.	109%	107%
	400 mg.	103%	108%
Morphine HCl:	11 mg.	103%	101%
	22 mg.	102%	105%
Diphenylethylamine HCl:	200 mg.	105%	108%

American authors have used this method extensively and successfully; the failure to confirm their results is disappointing. They have usually tested morphine after parenteral administration, but successful results with non-opiate drugs given by mouth have been reported, for instance by Wolff, Hardy & Goodell (1941).

The higher dose of morphine which we used was sufficient to produce definite nausea. The subjective effects of 200 mg. of diphenylethylamine were reported independently by both subjects to resemble those of mild drunkenness.

TOLERANCE TO ELECTRIC SHOCK IN RATS

Method

The method of Sivadjian (1935) was used. A box is floored with parallel copper wires, alternate wires being connected to the positive and negative terminals of the secondary coil of a Du Bois-Reymond apparatus. A male (90–110 g.) rat is placed in the box and the distance between the primary and secondary coils of the apparatus is decreased until the rat jumps when the current is switched on. Readings are taken at 15 min. intervals. Cross-over tests have been carried out on groups of five or six rats, of which three are injected subcutaneously with the test compound, and the remainder with a similar volume of physiological saline on one day and given the reverse injections on the following day. Two control readings are made before the injection is given, and readings are continued for 1–2 hr. afterwards.

Results

Both morphine and pethidine hydrochlorides produced significant increases in pain threshold, but none of the diphenylethylamine compounds did so. In view of this failure no attempt was made to calibrate the apparatus. The

results obtained with the hydrochlorides of morphine and pethidine are graphed in Fig. 1. The graphs indicate that with the increasing dose the rise

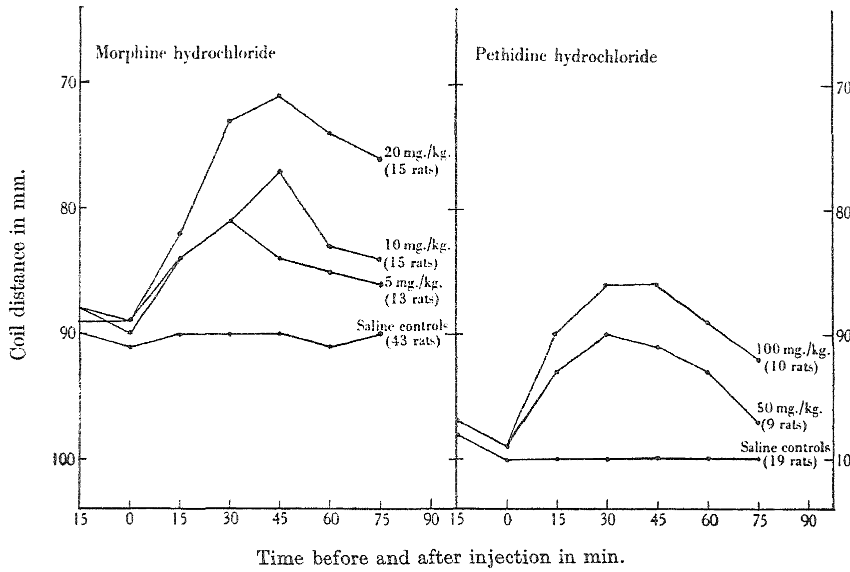

Fig. 1.

TABLE 2. Pain thresholds to electric shocks in rats

Test substance	Dose mg./kg.	No. of rats	Pre-injection reading*	Min. post-injection reading*	Max. fall
Morphine HCl	5	13 exp.†	89	81	8
		11 con.	90	89	1
	10	15 exp.	90	77	13
		15 con.	91	90	1
	20	15 exp.	89	71	18
		15 con.	90	89	1
Pethidine HCl	50	9 exp.	99	90	9
		9 con.	100	100	0
	100	10 exp.	99	86	13
		10 con.	100	99	1
β-Hydroxy-α:β-diphenyl-ethylamine HCl	50	5 exp.	85	86	—
		5 con.	83	81	2
	100	5 exp.	85	81	4
		5 con.	86	84	2
	200	10 exp.	101	96	5
		10 con.	102	101	1
	400	6 exp.	100	92	8
		5 con.	102	102	0

* Distance between coils in mm.
† exp. = experimental rats; con. = saline-control rats.

in pain threshold is increased so that the method may be capable of being adapted for quantitative assays.

In Table 2 are given the individual results with the hydrochlorides of morphine, pethidine, and β-hydroxy-α:β-diphenylethylamine. This last compound had given the most encouraging results in the clinical trials mentioned above and was therefore tested thoroughly. The drop in coil distance certainly increased with the dose, but the increases are hardly significant, certainly do not approach those attained with the other two compounds, and are suspect owing to the toxic symptoms produced with the higher doses (muscular tremor with 200 mg./kg., and convulsions in some of the rats injected with 400 mg./kg.).

The essentially negative results obtained with this compound were duplicated when the hydrochlorides of the related compounds listed in Table 3 were tested. The doses were the greatest that could be given without producing toxic symptoms. None of the compounds gave a fall in coil distance greater than has been obtained with saline controls in a group of the same number of rats.

TABLE 3. List of compounds tested with negative results

Compound*	Dose (mg./kg.)
αβ-Di(*p*-anisyl)ethylamine (M1)	100
αβ-Diphenyl-ethylene-diamine (M2)	500
αβ-Diphenylethylamine (M3)	100
β-Hydroxy-αβ-diphenyl-*n*-propylamine (M5)	100
β-Hydroxy-αβ-diphenyl-*n*-butylamine (M6)	100
Dimethylamino-benzyl-phenyl-ketone (M7)	50
β-Hydroxy-αβ-diphenyl-*n*-propyl dimethylamine (M8)	100
β-Hydroxy-αβ-diphenyl-*n*-butyl dimethylamine (M9)	100
α-(*p*-Hydroxyphenyl)-β-phenyl-ethylamine (M15)	50
α-Phenyl-β-*cyclo*-hexyl-ethylamine (M16)	50
	100
α-(*p*-Anisyl)-β-*cyclo*-hexyl-ethylamine (M17)	50
	100
α-*cyclo*-Hexyl-β-phenyl-ethylamine (M18)	50

* Each compound was tested as hydrochloride, and the laboratory numbers in brackets correspond to those in Dodds *et al.* (1944).

DISCUSSION

This failure to demonstrate analgesic activity in the diphenylethylamine compounds only applies to one form of painful stimulus, but the fact that pethidine gives satisfactory results does not suggest that the method of testing is at fault. Clinical trials by other workers not yet reported have confirmed our own observation that β-hydroxy-α:β-diphenylethylamine (M4) will relieve the particular pain associated with pressure on nerve caused by malignant growths, but have demonstrated little or no analgesic action on other more generalized types of pain. The reason for this apparently exclusive type of analgesic action may be related to an inhibition of nerve conduction and is being investigated.

SUMMARY

1. No significant rise in the pain threshold to heat in man was produced when 22 mg. of morphine hydrochloride or 200 mg. of diphenylethylamine were given by mouth. The latter administration produced subjective symptoms of mild drunkenness.

2. A method of measuring the pain threshold to electric stimuli in rats is described. Significant rises in threshold were produced by the hydrochlorides of morphine (5–20 mg./kg.) and pethidine (50–100 mg./kg.), but not by the hydrochlorides of diphenylethylamine and related compounds.

We are grateful to I. A. Hepple for technical assistance, and to Boots Pure Drug Co. for a supply of α-hydroxy-α:β-diphenylethylamine.

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Note added in proof (30 *Jan.* 1945). Subsequent tests have shown that mild analgesics (aspirin and antipyretic compounds of the phenetidine series) do not affect the tolerance of rats to electric shocks as determined by the method used above.

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THE BEHAVIOUR OF T. 1824 (EVANS'S BLUE) IN CIRCULATING BLOOD AND A MODIFIED METHOD FOR THE ESTIMATION OF PLASMA VOLUME

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(*Received* 24 *October* 1944)

In all determinations of the circulating plasma volume by dye methods, it is necessary to allow sufficient time to elapse for complete mixing of the dye with the plasma before withdrawing a sample for estimation of the concentration. During the mixing period a certain amount of dye disappears from the circulation, and in order to try to arrive at a value for the mixing time and to determine the loss of dye during this period, dye-disappearance curves have been plotted.

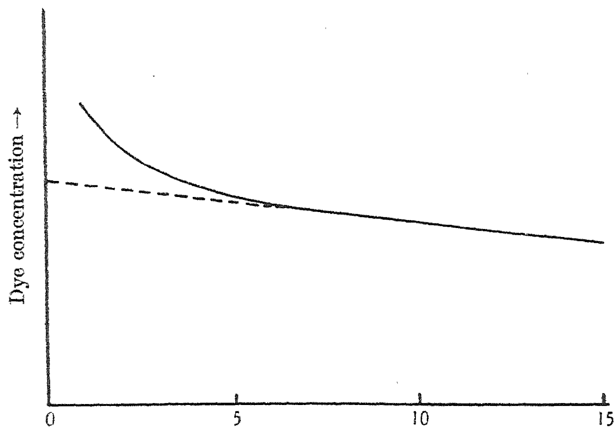

Fig. 1. General form of the dye disappearance curve of T. 1824 in circulating plasma, showing method of extrapolation to obtain initial dye concentration.

Gibson & Evans (1937), using the dye T. 1824, found disappearance curves which consisted of two distinct parts, an initial rapid fall in concentration followed by a slower loss which was nearly constant over a short period. The general form of these curves is shown in Fig. 1. The first part of the curve

was considered to be due to mixing and was termed the 'mixing curve'; the second part, due to disappearance of dye from the circulation, was called the 'disappearance slope'. The tangent point of these two curves, they considered, constituted the time of complete mixing, the 'mixing time'. Values of the order of 6 min. were obtained for the mixing time. The initial dye concentration was found by extrapolating the disappearance slope to zero time.

This determination of the mixing time has been widely accepted (Kennedy & Millikan, 1938; Courtice, 1943), but has been questioned by Robinow & Hamilton (1940). These last workers, using a continuous sampling method, observed fluctuations in the very early part of the curve which they thought probably indicated that mixing was complete in about 1 min. The method has also been criticized by King, Cole & Oppenheimer (1943), who pointed out that the tangent point of the two curves varies with the time range and, therefore, cannot be accepted as a criterion of complete mixing.

It appeared to us that the extent of that part of the curve generally accepted as indicating mixing was too great and too variable to be descriptive of this process, and that the initial fall represented a rapid disappearance of dye from the circulation. In order to get more conclusive evidence of the mixing time, and hence throw some light on the significance of the initial rapid phase of the curve, we have adopted a new method. Until mixing is complete, samples taken from different parts of the body should have different concentrations of the dye, whereas once mixing is complete the concentrations of dye in samples taken from any part should be equal. We therefore took simultaneous samples from jugular and femoral veins at intervals of 15, 30 and 60 sec. after injection of the dye and thereafter at longer intervals. The results of the investigation have led us to put forward an improved method for the estimation of blood volume by the use of T. 1824.

THE METHOD

A cannula was inserted into the right posterior facial vein of the cat so that its tip just entered the right external jugular without obstructing the blood flow. Heparin was injected through this cannula, 100 units/kg. of body weight. After allowing a few minutes for mixing, a sample of blood was withdrawn for use as a blank. Then 0·5 c.c. of a 1/2000 solution of T. 1824 per kg. of body weight was injected into the left jugular vein by means of a needle. The injection occupied about 10 sec. and the time at which half the dye had been injected was taken as zero time.

At short intervals, 1·5 c.c. samples of blood were withdrawn from the jugular vein and, simultaneously, samples of blood were taken from the femoral vein with a needle. As the needle tended to obstruct the free flow of blood in the vessel, these samples were taken alternately from opposite femorals to allow time for re-establishment of the circulation before the next sample was taken. All samples were drawn into oxalated tubes, centrifuged, and read directly in micro-cells with a Hilger absorptiometer, a red filter (Ilford 608) being used.

RESULTS

Typical results are shown in Fig. 2. It will be seen that, far from taking 6 min., mixing is complete in between 0·5 and 1 min. In a number of experiments we have found that, while the time taken for complete mixing varies slightly, it is always less than 1 min.

It appears, therefore, not only that the mixing time of 6 min. adopted by most workers is much too long, but that the rapid fall of concentration between 1 and 6 min. cannot be interpreted as a mixing curve. Furthermore Courtice (1943), while retaining a mixing time of 6 min., found that in some

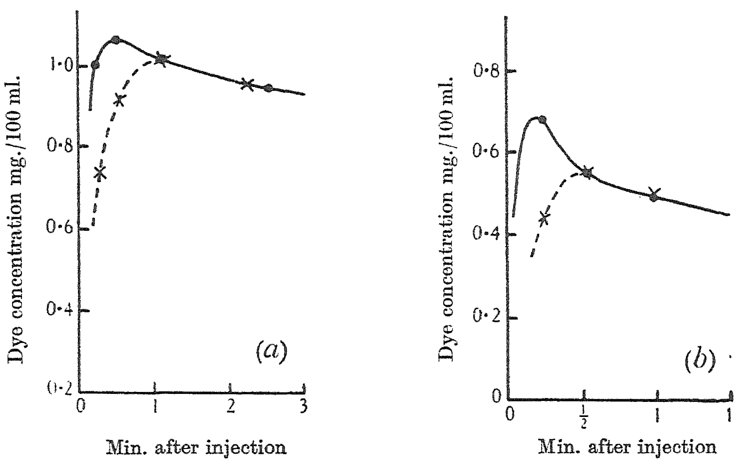

Fig. 2. Typical curves showing time taken for complete mixing of T. 1824 in the circulation of the cat, as indicated by the point at which dye concentrations in the jugular and femoral veins become identical. (*a*) Mixing complete in 1 min. (*b*) Mixing complete in ½ min. —— Jugular. ---- Femoral.

species the curve did not flatten out until a very much longer period had elapsed, certainly longer than could be necessary for mixing. Robinow & Hamilton (1940), following Smith (1925), suggest that the fall is due to loss into the lymph, but this appears improbable in view of the work of Courtice (1943).

We suspected that the so-called 'mixing curve' might be due to the absorption of the dye by some system which becomes saturated fairly rapidly. We therefore tried the effect of injecting a second dose of the dye after about 20 min., and determined the new disappearance curve. The rapid fall was completely absent from this, the curve being virtually linear from 1 min. onwards (Fig. 3).

The amount of dye taken up by the absorbing system is practically independent of the concentration of dye in the plasma. This is shown in Fig. 4. In curve (*a*) the concentration of dye was ten times that in curve (*b*). By

producing the 'disappearance slopes' backwards and subtracting the ordinates, curves are obtained showing the way in which the dye is taken up by the absorbing system alone (Fig. 4 *c*, *d*). It can be seen that the amounts of dye taken up are only in the ratio of 1·5 : 1 (within the limits of individual variation) in spite of the large difference in concentration.

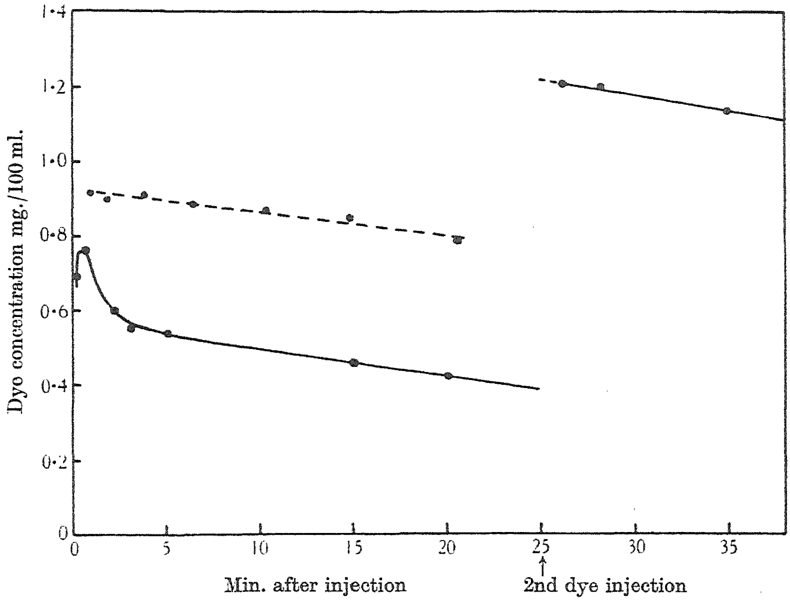

Fig. 3. Two successive doses of dye were injected with an interval of 25 min. The initial rapid fall in concentration present after the first injection is absent from the second curve. When Indian ink is previously injected the rapid phase is absent, even from the first curve (broken line). —— Normal animal. ---- Reticulo-endothelial systeme blocked.

The curves obtained in this way are exponential in form, and indicate that the absorption reaction is of the first order with respect to the absorbing system, being independent of the dye concentration, provided this is not very low ('pseudo-unimolecular reaction'). If this relation is correct then the curve should fit the equation,

$$K = \frac{1}{t_2 - t_1} \log_e \frac{x_1}{x_2},$$

where K is the velocity constant, and x_1 and x_2 represent the difference of the ordinates (i.e. the amount of the system remaining unsaturated) at time t_1 and t_2 respectively. The plot of $\log_e (x_1/x_2)$ against $t_2 - t_1$ should therefore be a straight line. The result of such a plot for a typical case is shown in Fig. 5.

King, Cole & Oppenheimer (1943) find that a single exponential equation of the form $c = c_0 e^{-Kt}$ will not fit the disappearance curve over an extended

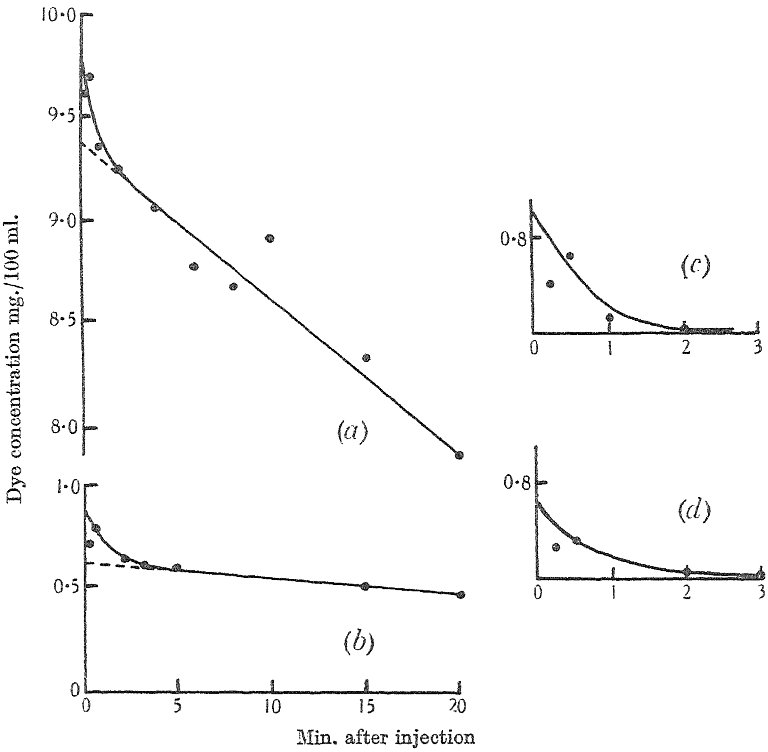

Fig. 4. (*a*) Initial dye concentration ten times that in (*b*). (*c*) and (*d*) are redrawn from (*a*) and (*b*) respectively with the 'disappearance slopes' as base lines, to show how the dye is taken up by the absorbing system alone.

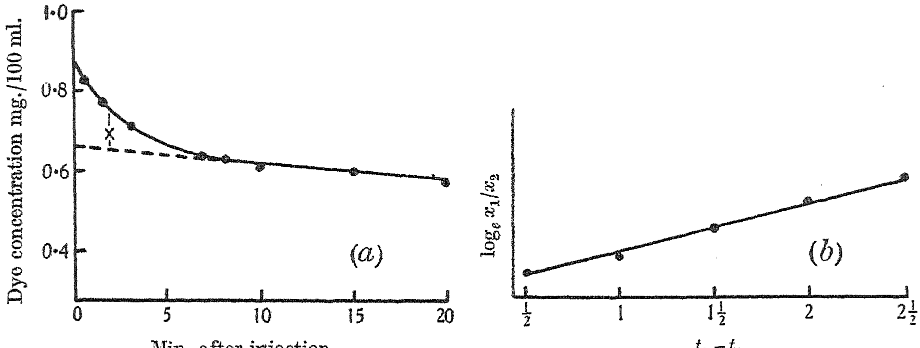

Fig. 5. (*b*) Straight line obtained by plotting values of $\log_e (x_1/x_2)$ against corresponding values of $t_2 - t_1$ for the curve (*a*).

period, and suggest empirically an equation of the form $c=c_0(1-\sqrt{(Kt)})$, which they find fits quite well, over a long period. That this equation cannot completely describe the curve is shown by their own admission that when t exceeds $1/K$ the relation breaks down and also, since the equation is that of a parabola, it implies, as Gregersen & Rawson (1943) have pointed out, that the rate of dye loss approaches infinity at the time of injection.

We have shown that two distinct mechanisms are involved in the disappearance curve, and it would therefore appear that at least two terms involving t are necessary to describe it. The first part of the curve (1–30 min.) is described well by the sum of an exponential and a linear expression. It does not appear that any simple expression can be found to fit the second term of the equation completely. Many attempts have been made, with more or less success, to describe the disappearance curve over a long period, in terms of time and concentration. It seems unlikely, however, that, once an appreciable fraction of the dye has left the circulation, the rate of disappearance would be solely a function of the dye concentration in the circulating plasma. Other factors, such as return from the lymph, rate of destruction of the dye, etc., must become important. However, we have demonstrated that when the dye-absorbing system has been saturated, the disappearance slope is virtually linear, at any rate during the first half hour, and hence, from a practical standpoint, may be extrapolated to obtain the initial concentration.

Similar results to the foregoing have been obtained by injecting the dye into the human subject (I. C. W.). The rapidly falling absorption curve was abolished in the same way after the first injection of dye (0·25 mg./kg.).

In order to determine the site of the absorbing mechanism we tried the effect of blocking the reticulo-endothelial system with Indian ink. Ordinary Indian ink was diluted with an equal volume of distilled water, and this solution was again diluted with its own volume of 0·9 % saline. The final solution was sterilized by boiling and centrifuged to remove the larger particles, which otherwise block the lung capillaries. It was given by intravenous drip into the femoral vein of the cat at the rate of about 3 c.c. per kg. of body weight.

After 24 hr. had elapsed the dye-disappearance curve was determined in the same manner as before. The initial rapid fall was absent from the curve (Fig. 3, broken line). Sections of the liver and spleen showed large amounts of carbon particles in the reticulo-endothelial cells. It appears, therefore, that blocking these cells abolishes the so-called 'mixing curve'.

Discussion

Gibson & Evans (1937) divided the curve obtained for disappearance of T. 1824 from the circulation into two parts, an initial 'mixing curve' lasting about 6 min. followed by a less rapid 'disappearance slope'. By determining the time at which the plasma dye concentrations in widely separated parts of the body become identical, we have shown that the mixing time is of the order of 1 min. The Gibson & Evans's so-called 'mixing curve' has thus no relation to mixing, and moreover it is abolished by a previous injection of dye or by blocking the reticulo-endothelial system with Indian ink. The disappearance curve thus actually consists of three parts, a 'mixing curve' lasting for about 1 min., followed by an 'absorption curve' lasting for about 5 min., the whole superimposed on a 'disappearance curve' ('disappearance slope' of Gibson & Evans) which can be plotted, uncomplicated by other effects, from 6 min. onwards. The time for which the absorption curve persists probably varies in different animals.

The 'mixing curve' varies in shape according to the relative positions of injection and sampling, and, in any case, is of such short duration that it can only be determined approximately. The 'absorption curve' is exponential in form when separated from the 'disappearance curve', the observed curve being the sum of the two. While an expression completely describing the 'disappearance curve' over long periods has not yet been put forward, the curve probably being a function of several variables interacting to different extents, it may be considered as linear over the first 30 min.

Since the 'absorption curve' represents actual removal of dye from the circulation and not mixing with plasma, as has previously been thought, it follows that extrapolation of the 'disappearance slope' to zero time will give an apparent value for the dye concentration which is too low, and hence the value for the plasma volume will be too high.

It is, of course, possible to determine the correct value by extrapolation of the 'absorption curve' or by calculation from the equation to this curve, but, to be reliable, both these methods require the determination of the curve with some accuracy and it is necessary to take a considerable number of samples at short intervals to do so. We suggest that a better method for routine determination is to abolish the absorption curve by injecting a 'saturating dose' of dye. After about 20 min. a blank is taken, an accurately measured dose of dye injected and the new curve determined. Since a straight line is then obtained over a period of 10–20 min., two or three samples are sufficient to determine it, and, by a simple extrapolation, the true initial dye value may be determined. The error by previous methods must vary with the concentration of dye in the plasma and possibly with the activity

of the absorbing system, and experiment shows that with concentrations of about 1 mg./100 c.c. the error may reach 20–30%.

It is interesting to note that previous workers have found that dye methods give higher results than other methods. Thus Hahn, Ross, Bale, Balfour & Whipple (1942) found that a 'tagged' red-cell method gave results about 25% lower than a method using vital red, while Smith (1925) quotes experiments in which the plasma volume was found to be about 16% lower by the carbon monoxide method than by vital red. Various explanations of the discrepancies have been put forward by these workers, but it seems possible that the same rapid absorption takes place with other dyes as with Evans's blue and may provide an alternative explanation.

The method here described gives, within very small limits of error, the absolute concentration of the dye.

It must be emphasized that these conclusions have been drawn from experiments on the normal animal and, as in most plasma volume studies by dye methods, their validity in abnormal circulatory conditions has not been established.

Summary

1. Mixing time for the dye T. 1824 with plasma is of the order of 1 min. in the cat.

2. The rapid fall in concentration between 1 and 6 min. is due, not to mixing, but to absorption of the dye by some mechanism, probably the reticulo-endothelial system, which becomes saturated by the end of this time.

3. The fall is abolished by previous injection of dye or of Indian ink.

4. Results of previous plasma volume determinations by the dye method are likely to be too high owing to loss of dye. It is suggested that a previous 'saturating' dose of dye should be injected before a determination is made, in order to abolish the 'absorption curve'.

We should like to thank Dr Fraser for valued assistance and also the Medical Research Council for a grant to one of us (E. W. H. C.) which defrayed the costs of this investigation.

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THE EFFECT OF CHRONIC ADMINISTRATION OF ADRENALINE ON THE SUPRARENAL CORTEX AND THE COMPARISON OF THIS EFFECT WITH THAT OF HEXOESTROL

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An investigation of the effect of chronic administration of adrenaline on the size and structure of the suprarenal glands was prompted by the observation (Vogt, 1944) that an injection of adrenaline greatly increases the output per minute of cortical hormone. The present paper contains a description of changes produced in the suprarenal cortex by repeated injections of adrenaline, and a comparison of the action of adrenaline with that of hexoestrol.

Babes & Jonescu (1908) claimed to have observed a 10–30-fold increase in the size of the suprarenals of rabbits after 14–20 intravenous injections of adrenaline, given over periods of 28–50 days. Kolmer (1918) was unable to confirm these claims in experiments on mice and guinea-pigs. Experiments on this subject were also carried out by Herrmann (1942); unfortunately, this paper was inaccessible to me.

METHODS

Two strains of rats were used for the experiments, albinos of the Wistar strain and piebald 'hooded' rats. The suprarenals of each rat, subjected to a particular treatment, were compared with those of an untreated litter mate of the same sex and, at least approximately, the same body weight. Statistical analysis could thus be carried out by the method for 'paired' experiments.

Adrenaline was injected under the skin of the neck or back 3–4 times daily (Sundays excepted) at intervals of 2·5–3 hr. The solutions were usually prepared from adrenaline base dissolved in the requisite amount of hydrochloric acid; the dilutions were made with 0·9% NaCl, and were kept in the refrigerator and frequently renewed.

At the end of an experiment, treated rats and controls were killed by a blow on the head. Where required, the heart was excised, dried on filter paper and weighed fresh. The suprarenals were dissected and fixed in formalin. After 24 hr., the glands were stripped of fat and connective tissue under the binocular microscope, weighed and embedded in gelatin. Frozen sections were made, and stained either with Sudan III and haematoxylin or with haematoxylin-eosin.

For hexoestrol treatment, a tablet of 250 mg., containing 1% stearic acid, was pushed into the loose subcutaneous tissue near the left scapula through a small midline incision. The skin was closed with sutures, and always healed by first intention. The implantation was done under ether anaesthesia.

Suprarenalectomies, using the dorsal approach, were performed with aseptic precautions under pentobarbitone-ether anaesthesia. The glands were immediately fixed in formalin. In those

animals which carried a hexoestrol tablet, this was removed together with the suprarenals through the same skin incision.

In order to denervate the suprarenals, use was made of the fact that all branches of the splanchnic nerves which supply the gland enter it along its oral edge and the upper part of its medial surface. Denervation can, thus, be performed by severing the loose tissue along a semicircular line starting lateral to the gland, then running parallel to its oral edge and finally bending in a caudal direction towards the hilum. Such a dissection also severs an artery accompanying the splanchnic nerves, but it was considered safer to sacrifice the artery than to risk incomplete denervation. The rats were anaesthetized with ether and tied on their backs to a warmed sloping board so that the head was lower than the abdomen. This position made the exposure of the glands through a ventral midline incision easier, since the liver was retracted by gravity from the upper pole of the kidneys. The intestines were wrapped in a piece of gauze and retracted sideways, and then the nervous and other connexions of each suprarenal were carefully cut under the binocular microscope at some distance from the gland itself along the line described above. The abdominal wound was sewn in two layers. Recovery of the rats was uneventful, except for a transitory loss of weight. Aseptic precautions were observed during the operations.

Hypophysectomies were carried out under ether anaesthesia by the usual parapharyngeal route. The rats were all albinos, but only some of them were litter mates. Completeness of the operation was checked under the dissecting microscope at the post-mortem examination.

Results

Normal rats

Adrenaline injections. In a first experiment, four Wistar rats (age 21 days, mean weight 33 g.) were injected twice and, later, three times daily with 25 μg. adrenaline-HCl. The controls received an equal number of injections of 0·9% NaCl. No pathological signs were observed during the first week. On the eighth day of the experiment, the dose of adrenaline was doubled; as a result, the rats died after the second injection from pulmonary oedema. The control members of the four pairs were then killed, and all the suprarenals weighed and examined histologically. Table 1 gives the figures for absolute and relative weights. Since the rats injected with adrenaline and the controls had grown at an equal rate, the result is the same whether actual or relative weights are considered.

TABLE 1. Rats of 21 days injected with adrenaline for a period of 8 days

		Mean weight of suprarenals	
	No. of rats	mg.	% body weight
Adrenaline-treated	4	14·75 ± 0·144	0·0350 ± 0·00196
Controls	4	14·80 ± 1·014	0·0348 ± 0·00168
Difference		0·05	0·0002

The figures in Table 1 leave no doubt that 8 days' treatment with adrenaline had not affected the weight of the suprarenals. Histological examination, however, revealed changes in the distribution of lipins. After treatment with adrenaline, the zona fasciculata, except for small patches, was stained with Sudan throughout its whole width, whereas the controls showed the usual picture of a comparatively fat-free inner third or half of this zone.

In a second experiment, treatment with adrenaline was more prolonged. Injections being again started at weaning time, the experiment could not be terminated before the rats had reached an age at which the sexual differences in relative suprarenal weight become manifest (Hatai, 1914). The results were, therefore, calculated separately for each sex. Injections were started at the age of 21–23 days (mean weight 44 g.) and continued for 24 days. Four doses of 25 μg. were administered daily, except for the last week, when 30 μg. were given.

From the end of the second week of treatment onwards, the rats receiving adrenaline were more excited than the controls; they were jerky in their movements and tried to escape when picked up; the nervousness was not evident at the first injection in the morning, but developed and increased during the day. This was an indication that the intervals of 2½–3 hr. between the injections were not sufficient for the elimination of the drug. During the 3rd week of the experiment, bald patches began to develop on the skin at the nape of the neck in some of the rats receiving adrenaline, whereas no damage to the skin was seen in the controls. By the end of the experiment, all adrenaline-treated animals exhibited some degree of baldness. Growth was slightly inhibited during the last week of treatment, so that the average gain over the whole period was 51 g. instead of the 57 g. seen in the controls.

In each pair of male or female rats, the glands of the animal subjected to adrenaline injections were heavier than those of the control. Owing to the slight check on growth during the last days of adrenaline injections, the differences of the actual weights are a little smaller than those of the ratio of gland weight to body weight. Nevertheless, the ratios are used in Table 2 since they are more informative for the comparison of animals of different sexes.

TABLE 2. Weights of suprarenals of Wistar rats as percentage of body weight after treatment with adrenaline for 3½ weeks

	Males treated with			Females treated with		
	Adrenaline	Saline	Difference	Adrenaline	Saline	Difference
	0·0226	0·0167	0·0059	0·0269	0·0230	0·0039
	0·0228	0·0172	0·0056	0·0236	0·0218	0·0018
	0·0233	0·0188	0·0045	0·0269	0·0242	0·0027
	0·0245	0·0162	0·0083	0·0240	0·0183	0·0057
				0·0241	0·0239	0·0002
Mean	0·0233	0·0172	0·0061	0·0251	0·0222	0·0029

The table shows that, at the age of 6–7 weeks, the relative weight of the suprarenals is considerably smaller in males than in females. This is the normal condition in adult animals. Both sexes have significantly heavier glands after adrenaline injections. The average gain is 0·0061 g./100 g. rat (or 35 % increase above normal) in the males, and 0·0029 g. (or 13 % increase above normal) in the females. Thus, in the does, which start at a higher suprarenal weight, there

is less increase in weight than in the bucks. The difference between the results in the two sexes is significant (Fisher's (1941) t-test for differences of means).

The histological findings are similar in kind, but more conspicuous than those seen after 1 week's treatment. Sudanophil substance is found throughout the whole zona fasciculata and zona reticularis, spreading centrally as far as the border of the medulla, and, peripherally, frequently invading the 'clear zone', a lipin-free band present in the normal gland below the zona glomerulosa.

Except for the bare patches of skin, nothing abnormal could be seen on post-mortem examination of the rats submitted to the adrenaline injections. When the hearts, however, were dissected and weighed, they were found, in each pair of rats, to be heavier in the adrenaline-treated member. The weight of the hearts was increased by an average of 0·0477 g./100 g. rat (standard error $\pm$ 0·00786), which is a gain in relative weight of 9·5 % above normal.

For any attempt at interpreting the excessive accumulation of lipins in the suprarenal cortex, it was desirable to know whether it was accompanied by such changes in the essential functions, or in the cortin requirements of the body, as would reveal themselves by an altered survival time after suprarenalectomy. An experiment was, therefore, performed on seven pairs of male Wistar rats (3–4 weeks old, mean initial weight 47·5 g.). Adrenaline and saline injections were made for 24 days, the doses being the same as in Exp. 2. On the 25th day, one injection only was given, after which all the rats were suprarenalectomized. The injections were now discontinued and the survival times of the rats observed. Four adrenaline-treated rats lived longer than their control litter mates, and three lived less long. The mean survival time was 7·4 days for rats to which adrenaline had been administered, and 7·0 days for the controls. The difference is obviously non-significant. There is thus no indication that adrenaline had caused any profound alteration in the rats' requirement of cortical hormone.

Denervation of the suprarenal glands. Since changes are produced in the suprarenal cortex by chronic administration of adrenaline, the question arose whether denervation of the glands would be followed by any structural alteration due to a chronically low level of adrenaline in the blood. Seven pairs of male rats, 70 days old, and belonging to the Wistar strain, were selected in order to test this point. The operated members of each pair were killed simultaneously with the controls, 14 days after denervation. The glands were excised, weighed, and examined histologically. Each denervated gland bore a small scar from an anaemic infarctation at its oral surface; this was due to the section of an artery taking the same course as the splanchnic nerves. The colour of the suprarenals was otherwise normal; the weights showed no consistent difference from the controls. In two instances, in which one gland had atrophied as a result of the operation, the other had hypertrophied. Except for the scar on the surface, the histological structure was unaltered

and the lipin distribution normal in the denervated glands. Hence, a low blood-adrenaline level due to bilateral splanchnotomy does not affect the morphology of the cortical tissue.

Implantation of hexoestrol tablets. Many investigators (Korenchevsky & Dennison, 1935; Noble, 1938*a*, *b*; Deanesly, 1939, etc.) have observed the hypertrophy of the suprarenal glands produced by female sex hormones and their synthetic substitutes. In the experiment reported in this section, the effects obtained with the synthetic oestrogen hexoestrol (di(*p*-hydroxy-phenyl)-*n*-hexane) are contrasted with those resulting from chronic administration of adrenaline. As in the previous experiments, observations were made of the size and the morphology of the suprarenals and of the length of survival time after suprarenalectomy, but the same animals were used for the different purposes. Hexoestrol (obtained through the courtesy of Dr S. J. Folley) was administered by subcutaneous implantation of tablets of 250 mg. The tablets were removed simultaneously with the suprarenals, and the glands were immediately fixed in formalin. Ten pairs of male rats (initial age 38–42 days and average weight 99 g.) and three pairs of female rats (age 60 days and average weight 134 g.), all of the piebald strain, were used. The tablets were removed after 10 days in the males and after 11 days in the females. As a result of this treatment, growth was completely arrested in the males, and the females lost an average of 12 g. body weight. During the same period, the control bucks gained 26 g. and the control does 29 g. At the operation, disappearance of the perirenal fat was observed in the animals treated with hexoestrol. The suprarenals were darker than usual, and hypertrophy was obvious on mere inspection. The weights are recorded in Table 3.

TABLE 3. Effect of implantation of hexoestrol tablets on the weight of the suprarenal glands of piebald rats

	Weight of suprarenals					
	Actual weight of both glands (mg.)			% body weight		
Sex	Treated rat	Control rat	Difference	Treated rat	Control rat	Difference
♂	34·6	20·9	13·7	0·0427	0·0211	0·0216
♂	34·4	26·8	7·8	0·0398	0·0285	0·0113
♂	32·4	29·5	2·9	0·0334	0·0242	0·0092
♂	55·8	27·4	28·4	0·0754	0·0214	0·0540
♂	37·6	27·8	9·8	0·0432	0·0220	0·0212
♂	44·5	26·4	18·1	0·0371	0·0203	0·0168
♂	31·0	30·4	0·6	0·0292	0·0232	0·0060
♂	32·3	25·4	6·9	0·0276	0·0184	0·0092
♂	29·7	18·9	10·8	0·0291	0·0151	0·0140
♂	40·0	35·2	4·8	0·0381	0·0246	0·0135
Mean	37·25	26·87	10·38	0·0396	0·0219	0·0177
♀	59·1	53·3	5·8	0·0473	0·0321	0·0152
♀	69·2	52·9	16·3	0·0563	0·0324	0·0239
♀	65·1	43·8	21·3	0·0551	0·0298	0·0253
Mean	64·5	50·0	14·5	0·0529	0·0314	0·0215

The first observation to be made from this table concerns the normal glands. In this strain of piebald rats, the suprarenals are larger than in the Wistar rats hitherto used. The mean suprarenal weight of ten normal piebald males at 50 days is $0{\cdot}0219 \pm 0{\cdot}00115\%$ of the body weight, whereas a corresponding figure for white rats was found to be $0{\cdot}0172 \pm 0{\cdot}00056\%$. The percentage for female piebald rats is $0{\cdot}0314 \pm 0{\cdot}00082$ at 70 days. The only observation on normal white females was made at 47 days and was $0{\cdot}0222 \pm 0{\cdot}00107\%$. Since the relative weight diminishes with age, the difference between the two strains is in fact larger than would appear from the available figures.

The effect of the tablets is quite uniform. Expressed as actual increase over the weight of the control glands, the glands of the males are 38·6 % and those of the females 29 % heavier after treatment. In percentage of body weight the corresponding figures are a rise of 81 and 68·5 % respectively (Table 4). The fact that the relative weights increase more than the absolute values is to some extent a result of the inhibition of growth by the hexoestrol, an effect first seen by Noble (1938*a*).

TABLE 4. Increase in weight of suprarenal glands

	Increase above normal resulting from treatment with			
	Adrenaline (24 days)		Hexoestrol (10–11 days)	
	♂	♀	♂	♀
Absolute weight	23·5 %	5·8 %	38·6 %	29·0 %
Percentage of body weight	35 %	13 %	81 %	68·5 %

In Table 4, the increase in suprarenal weights is contrasted with that obtained from adrenaline injections administered over a period of 24 days. After implantation of hexoestrol tablets, the enlargement of the suprarenals exceeds, in both sexes, that obtained with adrenaline. The difference between males and females in the degree of the response, though significant in the experiment with adrenaline, is not so in the experiment with hexoestrol.

The histological picture of the gland after hexoestrol treatment is fundamentally different from that of rats subjected to adrenaline. Not a granule of fat is present in any part of the cortex. The cells are enlarged, the cytoplasm and nuclei appear swollen, and in a few instances recent haemorrhages into the tissue are found. Loeser (1939) was the first to observe the disappearance of lipins from the suprarenal cortex as a result of injecting *stilboestrol* into rats. A necrotic zone, however, like that seen by McPhail & Read (1942) in female mice, was not present in either sex in Loeser's or in the present experiments.

There is evidence, again in contrast to the conditions produced by chronic administration of adrenaline, that profound alterations in the resistance of the rats accompany this type of hypertrophy of the suprarenals. The rat with the largest increase in suprarenal weight (more than double that of the control)

showed the greatest loss in body weight occurring amongst the males, and died from the intraperitoneal injection of a quantity of pentobarbitone amounting to half the normal anaesthetic dose. The glands contained haemorrhages, and this may have accounted for the poor health of the animal. Haemorrhages, however, were the exception in these rats, and yet, after suprarenalectomy, the survival times were shorter than in the controls in all but two of the hexoestrol-treated rats. On the average, the implantation of hexoestrol reduced the survival time of the rats by 3·1 days (from 8·5 to 5·4 days). This difference of the means is highly significant.

Some information about the speed at which the changes due to hexoestrol develop was obtained from a few bucks which were killed as early as five days after the implantation. The mean increase in glandular weight per 100 g. rat was only 19%, but the histological picture was greatly modified: the zona fasciculata and zona reticularis were completely devoid of lipoidal material, whilst the cells in the zona glomerulosa were so packed with fat droplets, that the zone formed a more compact ring of sudanophil tissue than is usual in the normal gland.

Hypophysectomized rats

The accelerated release of cortical hormone which follows a single infusion of adrenaline is independent of the presence of the pituitary, whereas the effects of oestrogens on the suprarenal are usually considered, at least in the mammal, to be mediated by the anterior lobe (Selye & Collip, 1936; Ellison & Burch, 1936; Golla & Reiss, 1941; Janes & Nelson, 1942; Long, 1942). Histological examination of lipin distribution has, however, apparently not been carried out on hypophysectomized animals given oestrogens. Furthermore, structural changes, not necessarily accompanied by increase in size, have been reported to result from testosterone injections in the hypophysectomized rat (Leonard, 1944). A few experiments were, therefore, performed on the effect, after hypophysectomy, of chronic adrenaline treatment on the one hand, and of hexoestrol administration on the other, with a view to finding out whether the lipin storage seen with the first and the loss produced by the second drug in normal rats were still obtainable. The suprarenals were weighed, their lipins stained, and the results compared with observations made on untreated controls killed the same number of days after hypophysectomy. In all treated rats, adrenaline injections were started or hexoestrol tablets implanted on the third post-operative day, when atrophy of the suprarenals has not yet developed and the response to adrenotrophic hormone is still normal (Sayers, Sayers, Fry, White & Long, 1944). Adrenaline treatment was continued for 11, 12 or 23 days. It was begun with three and soon increased to four injections per day, the dose rising from 25 to 40 or 50 μg. at each injection. Baldness and excitability were encountered, as in normal rats, when the treatment was sufficiently prolonged.

No increase occurred in the suprarenal weights. Whether adrenaline had been given or not, they fell to less than 0·010% body weight by about the seventh post-operative day (normal figure 0·017%). The cortical lipins of controls and of injected animals alike had accumulated, as is known to occur in untreated hypophysectomized rats, in the zona glomerulosa, in the inner zona fasciculata and in the zona reticularis, leaving a wide ring of outer zona fasciculata entirely fat-free. A further accumulation of lipins in the layers adjoining the medulla, as was shown above to follow adrenaline treatment in the normal rat, was, evidently, hardly possible, but neither was there any change in the structure of the outer zona fasciculata, which was just as devoid of lipins with adrenaline administration as without.

The action of hexoestrol was examined 4, 5, 10 and 11 days after implantation. No increase in suprarenal weight was recorded, nor was there any disappearance of fat from the layers where it is found in the untreated hypophysectomized rat. The sections were in every respect indistinguishable from those of the hypophysectomized controls.

Discussion

Chronic administration of adrenaline to rats in doses which, in spite of their causing mild cardiac hypertrophy, do not grossly impair the health of the animals, produces an increase in suprarenal size accompanied by conspicuous changes in the distribution of the cortical lipins. Before attempting an interpretation of this effect, it will be useful to contrast it with the observations made on the suprarenal hypertrophy which follows treatment with the synthetic oestrogen hexoestrol.

The increase in suprarenal weight due to adrenaline is smaller than that resulting from hexoestrol; only following adrenaline is it significantly larger in males (which always have the smaller glands) than in females. After adrenaline, it is accompanied by a storage of lipins in all layers of the cortex, including those which normally contain little or no fat; after hexoestrol, complete disappearance of the lipin stores of the glands ensues. Whereas the survival times of rats, which are suprarenalectomized immediately after intensive treatment with adrenaline, is the same as that of normal controls, hexoestrol-treated rats die more rapidly after suprarenalectomy.

Lipin storage in the suprarenal cortex has been interpreted as a sign of increased function by some authors (Flexner & Grollman, 1939), and of decreased activity by others (Dosne & Dalton, 1941). A response which is apparently very similar to that produced by chronic adrenaline injections was obtained by Knouff, Brown & Schneider (1941) in guinea-pigs, exhausted by running in a treadmill. On account of the short duration of the experiments (5–9 hr.), it is doubtful whether it is permissible to suggest that secretion of adrenaline was causing the changes observed in the distribution of cortical lipins in these experiments on exhausting muscular exercise, but the point

could probably be settled experimentally. Zwemer (1936) describes a reaction of the suprarenal which he calls the 'lipoid storage type', and considers it to be the result of mild stimulation of the cortical cells (for instance by thyroid administration), whereas the histological picture of a hyperplastic, vascular and lipin-depleted gland is attributed to 'prolonged', or to 'severe, prolonged' activity. Evidently, the 'lipoid storage type' recalls the picture described above as the result of treatment with adrenaline, and the 'prolonged activity' type that following hexoestrol. It would be pure speculation to try and tell, from the histological picture of a gland, how much hormone it has recently released into the circulation, but some information may be obtained from certain physiological observations: on the one hand, we know from acute experiments that an injection of adrenaline increases the output of cortical hormone in the whole animal and in the perfused gland (Vogt, 1944 and unpublished). The gland which, after repeated injections of adrenaline, contains lipins in nearly every cortical cell can, therefore, not be a resting gland, neither is there any indication from the health of the animals, or their survival time after suprarenalectomy, that it is a damaged gland. The hexoestrol treated rats, on the other hand, are, despite their very large suprarenals, in a poor state of health; furthermore, death, interpreted as due to suprarenal deficiency, has been observed to follow stilboestrol administration in mice (McPhail & Read, 1942). Whatever, therefore, the functional capacity of the glands might be, they are apparently unable to cope with the demands of the oestrogen poisoned organism. It is even conceivable that the function of these hyperplastic suprarenals is severely deficient, like the function of the hyperplastic thyroids of animals given sulphaguanidine or similar compounds. Both the proliferating effect of sulphaguanidine on the thyroid (MacKenzie & MacKenzie, 1943; Astwood, Sullivan, Bissell & Tyslowitz, 1943), and of hexoestrol on the suprarenal, disappear after hypophysectomy. In the case of the thyroid, the hyperplasia appears to be the result of increased hypophyseal activity, caused by insufficient secretion of the peripheral endocrine gland, but there is no proof that a similar mechanism holds for the suprarenal hypertrophy. On the contrary, the observation made by Janes & Nelson (1942), that stilboestrol causes pituitary enlargement in the suprarenalectomized rat, might be considered as evidence for an interpretation which attributes the effect of this drug to a primary action on the pituitary.

A peculiar histological picture is given by the suprarenals of rats which have been submitted to the influence of hexoestrol for five days only. Whereas the zona fasciculata and reticularis have entirely lost their lipins, the zona glomerulosa contains even more fat than usual. This finding recalls the observation by Calma & Foster (1943), that trypan blue, when injected into rats, is stored exclusively in the glomerular layer. Such phenomena are difficult to understand, unless we assume that the different layers have

different functions and, therefore, do not respond in the same manner to the same stimulus.

The absence of any response to prolonged administration of adrenaline by the suprarenal of the hypophysectomized rat can be explained as the result of the degenerative changes occurring in the glands through lack of corticotrophic hormone, or by assuming that the response in the normal animal is mediated by the anterior lobe. In view of the fact that a single infusion of adrenaline causes increased cortical secretion in the absence of the pituitary, mediation by the anterior lobe would require the postulation of a different mode of action for the same drug, according to whether it is given once or injected repeatedly.

The lack of any lesions or abnormalities in the cortex of the denervated suprarenal is in good agreement with the absence of any sign of cortical deficiency in animals without suprarenal nerves, and with the interpretation of the effect of adrenaline on the cortex as merely an emergency response to sudden stress or strain.

Summary

1. Injections of adrenaline into young rats, repeated 3 times daily for 8 days, cause an accumulation of lipins in the suprarenal cortex.
2. If the treatment is prolonged for 24 days, the lipin storage becomes greater still and is accompanied by a moderate, but significant increase in suprarenal weight. The increase is twice as large in males as in females in spite of the fact that the glands of normal bucks are smaller than those of normal does.
3. The response cannot be obtained after hypophysectomy.
4. Suprarenalectomy, carried out at the end of prolonged adrenaline administration, is survived for the same length of time as without a preliminary injection period. This seems to indicate that no change has occurred in the animals' requirement of cortical hormone, and that their health has not been severely damaged by the treatment.
5. The suprarenal hypertrophy produced by implantation of hexoestrol tablets leads to complete loss of cortical lipins within 10 days. If treatment is restricted to 5 days, sudanophil granules are found in the zona glomerulosa but nowhere else.
6. After administration of hexoestrol, the rats do not survive suprarenalectomy as long as the controls. It is, therefore, doubtful whether the hyperplastic glands are able to secrete normally.
7. No structural changes are produced by hexoestrol in the suprarenal of the hypophysectomized rat.
8. Denervation of the suprarenals does not cause any change in their lipin content or in their size.

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THE INFLUENCE OF DURATION OF SENSITIZATION ON ANAPHYLAXIS IN THE GUINEA-PIG

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It has long been known, from the results of numerous workers, that the amount of protein required, in the reacting injection, to induce anaphylactic shock in the sensitized guinea-pig, is smaller in animals which have been sensitized for a long period, than in those tested soon after they become sensitive. No investigation appears to have been made of whether the amount of antigen needed to desensitize the tissues varies with the duration of sensitization, with the exception of some experiments on serum-sensitized guinea-pigs (Winter, 1944*a*).

In these experiments, it was found that the amount of globulin required to desensitize the uterus *in vivo*, when the reacting dose was injected into the portal blood, was greater in animals sensitized for 50 days than in animals which were examined 15–20 days after sensitization. There was also a greater tendency to shock, after the injection of a small amount of globulin, in the 50-day animals. In the present work this study has been extended, to include globulin-sensitized and albumin-sensitized animals, to maintain the investigation for a longer period, and to determine whether the increase in the amount of antigen required to desensitize the animal, as sensitization advances, is peculiar to serum-sensitized guinea-pigs alone. The point is of some theoretical interest, since it is well known that animals, sensitized by a single protein, which have recovered from anaphylactic shock induced by this protein, cannot be thrown into shock by another, even much larger, injection of the same antigen given shortly after recovery from shock. A similar finding applies to smooth muscle; it was shown by Dale (1912), and by Dale & Hartley (1916), that when a full reaction has been elicited from the isolated uterus of the sensitized guinea-pig, the further introduction of antigen to the bath causes no response from the muscle. It is remarkable, therefore, that in the serum-sensitized guinea-pig the amount of globulin needed to cause anaphylactic shock should decrease with the longer time of sensitization, while the capacity of the serum-antibody in the whole animal to fix globulin should increase.

Methods

Three groups of forty-eight virgin guinea-pigs were used in succession, the largest number which it was possible to accommodate at one time. The animals weighed between 200 and 250 g. on the date of sensitization. The groups were sensitized with horse serum, albumin and globulin respectively, the method of sensitization and the preparation of the proteins used being the same as in the previous paper (Winter, 1944*a*). Some animals from each group were tested when they first became sensitive, the serum-sensitized and globulin-sensitized guinea-pigs 15–18 days after injection and the albumin-sensitized animals after 28–30 days. Other animals from each group were tested after 48–51 days and the remainder after 90–93 days. The general principle of all the tests was the same; each animal was anaesthetized with ether, the first uterine horn was

removed and tested in the bath for sensitivity, 0·1 c.c. of horse serum being used in every case. The reacting dose of antigen was injected, into the portal blood unless otherwise stated, and the animal was killed 1–2 hr. later, when the second horn was tested in the bath. When, owing to the susceptibility of the animals to shock, the reacting dose of protein had to be given in more than one injection, the details are given in the protocols, but in all cases the animals were killed 1–2 hr. after the last injection. One preparation of globulin was used as the reacting antigen for the serum-sensitized and globulin-sensitized animals; the albumin-sensitized guinea-pigs were injected with albumin. The albumin and globulin, dissolved in 0·9% NaCl, were used in three concentrations: for injections of 1·0 mg./100 g. and over, 10 mg./c.c.; for injections smaller than 0·05 mg./100 g., 1·0 mg./c.c.; for all other injections, 5 mg./c.c.

Results

Animals sensitized by albumin

In the earlier work (Winter, 1944*a*) no symptoms of shock were observed in any albumin-sensitized animal which was given a reacting dose of albumin, but in those experiments the minimum amount of albumin necessary to desensitize the uterus was not exceeded. The present series (Table 1) shows that, with approximately twice the minimum amount of albumin, shock may

Table 1. Animals sensitized by albumin

mg. albumin per 100 g.					
1st	2nd	Total	Days sensitized	Reaction of 2nd horn	Remarks
.	.	0·1	28–30	+ − + +	No reaction
.	.	0·25	28–30	− − − +	No reaction
.	.	0·5	28–30	− −	No reaction
.	.	0·5	28–30	.	Two animals. Death in shock 4–5 min. after injection. 2nd horns not examined
.	.	0·25	49–50	+ + + +	No reaction
.	.	0·5	49–50	− − −	No reaction
.	.	0·5	49–50	.	Two animals. Death in shock 4–5 min. after injection. 2nd horns not examined
0·05	0·15	0·5	49–50	− − −	1st injection given. 1st horn removed. 2nd injection given. 3rd injection 2½ hr. later
.	.	0·5	91–93	.	Four animals. All died about 5 min. after injection
0·05	0·15	0·5	91–93	−	1st injection given. Moderate shock during removal of 1st horn. 2nd injection given. 3rd injection 3 hr. later
* 0·025	0·1	0·5	91–93	−	Injections as above. No reaction
* 0·025	0·1	0·5	91–93	− −	Injections as above. Moderate shock in one animal, severe in the other, after 1st injection
* 0·025	.	.	91–93	.	Two animals. Death 4–5 min. after injection
* 0·025	0·1	.	91–93	.	Death 5 min. after 2nd injection
0·005	0·02	0·25	91–93	− − + + +	1st injection given. 1st horn removed. 2nd injection given. 3rd injection 3 hr. later. 1st animal suffered severe shock after 2nd injection

* Group A (see text).

occur in some animals soon after they become sensitive. The susceptibility of the 50-day albumin animals to shock was no greater than that of those sensitized only for 28 days, but the amount of albumin required to desensitize the uterus had increased. With still longer time of sensitization the 90-day guinea-pigs showed symptoms, or died in shock, after a much smaller amount of albumin than was required to cause shock in the 50-day animals; on the other hand, although the susceptibility of the 90-day animals to shock had increased, there was no increase, or even a slight decrease, in the amount of albumin required to saturate the albumin-antibody in the whole animal.

Results obtained from some of the guinea-pigs sensitized 90 days with albumin bear closely on previous experiments (Winter, 1944*b*), which showed that the first uterine horns were insensitive in a number of animals in which anaphylactic shock occurred. Six animals in Table 1, marked group A, were injected with albumin, 0·025 mg./100 g.; two died in shock. The remainder received 0·1 mg./100 g.; one died, and two others showed moderate or severe symptoms of shock. Only one of the six guinea-pigs showed no symptoms. Four other animals were given 0·025 mg./100 g., followed, after removal of the first uterine horn, by 0·1 mg./100 g. The first uterine horns of these animals proved to be insensitive. No symptoms of shock were observed in these guinea-pigs, although they received the same sensitizing injection at the same time as the others. It is unlikely, therefore, that these insensitive uteri were in fact sensitive, and merely failed to react when tested in the bath, through inability of the antigen, for some reason, to enter the muscle.

Animals sensitized by serum

No symptoms of shock were observed in any 15–17-day serum-sensitized guinea-pig given a reacting dose of globulin in amount considerably greater than that necessary to desensitize the animal. Sensitiveness to a small dose of globulin was apparent in the 50-day animals, as was also the increase in the amount of protein required to desensitize the uterus. The experiments with 15- and 50-day serum animals in Table 2 are therefore confirmatory of the earlier results. Extension of this series to 90 days showed that there was a further increase in the amount of globulin required to saturate the serum-antibody in the tissues. A few 90-day guinea-pigs were still available, and these were given injections of globulin into the systemic circulation; the results show that the increase in the amount of globulin required to desensitize the animals, when they are kept for a long period, is not entirely to be ascribed to increased fixation of the antigen by cells in the liver. Although it is doubtful whether there was any significant change in the susceptibility of the serum animals to shock between 50 and 90 days, it is noteworthy that none of the 90-day animals which received the globulin into the systemic circulation showed any symptoms.

TABLE 2. Animals sensitized by serum

mg. globulin per 100 g. 1st	Total	Days sensitized	Reaction of 2nd horn	Remarks
.	0·05	15–17	– + – +	No reaction
.	0·1	15–17	+ – –	No reaction
.	0·5	15–17	– – – – –	No reaction
.	0·5	50–51	–	No reaction
.	0·5	50–51	+	Died 3 min. after injection
.	0·5	50–51	+	Severe shock; recovered
0·15	.	50–51	.	Died 4 min. after injection. 2nd horn not examined
0·15	0·5	50–51	– + – +	1st horn removed. 1st injection given. 2nd injection 3 hr. later
0·15	1·5	90–93	– – –	Injections as above
0·15	1·5	90–93	–	Injections as above. Moderate shock after 2nd injection; recovered
0·15	.	90–93	.	Died 4 min. after injection
0·15	0·5	90–93	+ + + +	1st horn removed. 1st injection given. 2nd injection 3 hr. later
0·15	0·5	90–93	+ –	Injections as above. Severe shock in both animals after 1st injection; recovered
0·15	0·5	90–93	– – + + + –	1st horn removed. 1st injection into inferior vena cava. 2nd injection 3 hr. later into jugular vein

Animals sensitized by globulin

Unlike serum-sensitized guinea-pigs, when animals sensitized by globulin first become sensitive, they are susceptible to anaphylactic shock after an injection of globulin insufficient to desensitize the uterus. This conclusion receives further support from the experiments recorded in Table 3, in which three of the seven animals given 0·5 mg. globulin per 100 g. died in shock. Animals kept for 50 days were more susceptible to the reacting protein, but no difficulty was experienced in administering the desired dose by graded injections. The amount of globulin necessary to desensitize the animals was not increased by the longer time of sensitization; in this respect, globulin animals differ from 50-day serum-sensitized guinea-pigs, but they resemble them in showing increased susceptibility to shock. The liability of the 90-day globulin animals to shock, following injection of globulin, was very much greater than at 50 days, and it was probably only by chance in the first two experiments (A and B) with the 90-day guinea-pigs, that the animals survived without shock, after the same series of injections which was successful in the last four 50-day animals. The next six guinea-pigs died in shock, although three received even smaller preliminary injections, and it seemed probable that the whole of the remainder would be lost without yielding more than a few isolated results. Experience with the 90-day serum-sensitized guinea-pigs, in which some of the animals suffered from shock after injection into the portal blood, while none of those injected into the jugular vein showed

TABLE 3. Animals sensitized by globulin

mg. globulin per 100 g.							
1st	2nd	3rd	4th	Total	Days sensitized	Reaction of 2nd horn	Remarks
.	.	.	.	0·25	16–18	+ +	No reaction
.	.	.	.	0·5	16–18	− + + +	No reaction
.	.	.	.	0·5	16–18	.	Three animals. Died 4–5 min. after injection. 2nd horns not examined
0·05	0·1	.	.	1·0	16–18	+ − + −	1st injection given. 1st horn removed. 2nd injection given. 3rd injection 3 hr. later
0·05	0·1	.	.	1·5	16–18	− − −	Injections as above
0·05	0·1	.	.	.	48–50	.	Death 5 min. after 2nd injection. 2nd horn not examined
0·05	0·05	.	.	1·5	48–50	− −	1st injection given. Moderate shock during removal of 1st horn. 2nd injection given. 3rd injection 3 hr. later
0·05	0·05	.	.	1·5	48–50	+ +	Injections as above. Animals died 5 min. after last injection
0·05	0·075	0·25	.	1·5	48–50	− − − −	1st injection given. 1st horn removed. 2nd injection given. 3 hr. later, 3rd injection given. Intestines replaced in abdomen for 5 min. Final injection given. Severe shock in one animal 6 min. after 2nd injection
0·05	0·075	0·25	.	1·5	90–93	− −	Experiments A, B. Injections as above
0·05	0·075	.	.	.	90–93	.	Experiments C, D, E. Three animals. Slight shock in two after 1st injection. All died 5–7 min. after 2nd injection
0·01	0·04	0·05	.	.	90–93	.	Experiment J. 1st injection given. 1st horn removed. 2nd injection given. 5 min. interval. 3rd injection given. Animal died 5 min. later
0·01	0·04	.	.	.	90–93	.	Experiments K, L. Two animals. Died 3–4 min. after 2nd injection
0·01	0·04	0·05	0·25	1·5	90–93	− −	Experiments N, O. 1st injection given. 1st horn removed. 2nd injection given. 5 min. interval. 3rd injection given. 3 hr. later, 4th injection given. 5 min. interval. Final injection given. Moderate shock in one animal after 2nd, in other after 3rd injection. 1st three injections given into inferior vena cava
0·05	0·075	.	0·1	0·5	90–93	+ −	Experiments F, G. 1st injection given. 1st horn removed. 2nd injection given. 2 hr. later, 3rd injection given. 5 min. interval. Final injection given. 1st two injections given into inferior vena cava
0·05	0·075	.	.	.	90–93	.	Experiment H. Animal died 8 min. after 2nd injection. Injections into inferior vena cava
0·05	.	.	.	.	90–93	.	Experiment I. Injection into inferior vena cava. Death 5 min. later
0·01	0·02	0·05	0·1	0·5	90–93	− − +	1st injection given. 1st horn removed. 2nd injection given. 5 min. interval. 3rd injection given. $2\frac{1}{2}$ hr. later, 4th injection given. 5 min. interval. Final injection given. 3rd injection given into inferior vena cava
0·01	0·04	0·05	.	0·5	90–93	+	Experiment M. 1st injection given. 1st horn removed. 2nd injection given. 5 min. interval. 3rd injection given. 2 hr. later, final injection given. 1st three injections into inferior vena cava

any reaction, suggested that the administration of some of the protein by the systemic blood might aid desensitization of the highly sensitive globulin animals. All the remaining 90-day globulin animals received some of the antigen into the inferior vena cava, and the series was successfully completed. The results show that there was no increase in the amount of globulin required to desensitize the animals after 90 days, but even a slight decrease. The variations in the susceptibility to shock in the three types of animal studied, at the different periods, and the amount of protein necessary for desensitization, are summarized in Table 4.

TABLE 4. Variations in susceptibility of animals sensitized with different antigens

	Duration of sensitization (days)		
	17 (28 for albumin)	50	90
Serum-sensitized animals			
mg. globulin per 100 g. required to desensitize	Below 0·5	Increased	Increased
Susceptibility to shock	None found	Moderate	Moderate
Globulin-sensitized animals			
mg. globulin per 100 g. required to desensitize	1·5	Not increased	Slightly decreased
Susceptibility to shock	Moderate	High	Very high
Albumin-sensitized animals			
mg. albumin per 100 g. required to desensitize	0·25	Increased	Slightly decreased
Susceptibility to shock	Moderate	Moderate	High

It is possible that the greater ease of desensitization, when some of the reacting protein was injected into the systemic blood, may be explained by the presence in the liver of sensitized cells which fix the antigen. It has already been suggested (Winter, 1944 *a*, *b*) that there is a differential distribution of the sensitizing antibody in the tissues of the guinea-pig, and in some animals the primary event in anaphylactic shock may occur in the liver. If this view is correct, there will be, when a very small amount of antigen is given, a greater tendency to shock when injection is made into the portal than into the systemic blood, but no difference will be observed when a larger quantity of protein is used, since, in the latter case, there will still be a sufficient concentration of antigen in the blood to affect the liver cells, should these be more highly sensitized than those in the smooth muscle of the lungs. The results obtained from the 90-day globulin animals support this view. If the five experiments A–E in Table 3 are compared with the four F–I, it will be seen that, with the larger dose of globulin, there was no significant difference between the susceptibility of the animals to shock. With the smaller dose, however, the three animals J–L, given the protein into the portal blood, all died; the three animals M–O, receiving it in the systemic circulation, all survived.

Shock in albumin animals after sensitization for 90 days

All the albumin-sensitized guinea-pigs recorded in Table 1 received the reacting dose of antigen in the portal blood. The results obtained from the highly sensitized serum and globulin animals, which showed a greater susceptibility to shock when a very small amount of antigen was injected into the portal blood, than when it was given into the systemic circulation, made it of interest to determine whether the same effect was shown in 90-day albumin-sensitized guinea-pigs. Twenty-four animals were given the usual preliminary injection of albumin in order to test this point. After 90–92 days, twelve were examined: all were sensitive, and the results were so convincing that the remainder were used for another purpose. In the first six experiments the very small dose of 0·025 mg. albumin/100 g. was injected, into the mesenteric vein or the inferior vena cava, in alternate animals: the first uterine horn was then removed and tested for sensitivity. The three guinea-pigs which received an injection into the portal blood all died in shock 3–4 min. later; of the three animals which received the antigen into the systemic blood, one died in shock, the other two showing no symptoms. An even smaller dose, of 0·015 mg./100 g. was used for the next six animals. Two of the three guinea-pigs given the injection into the portal blood died in shock; none of those which were given the albumin into the inferior vena cava showed any reaction. When, however, the five animals which survived the small injection of albumin into the inferior vena cava without symptoms, received, by the same route, 5 min. after the first injection, a further injection of albumin, 0·05 mg./100 g., three died in shock 3–4 min. later. There is, therefore, as was found in the case of the globulin-sensitized animals, no significant difference between the results obtained on injection by either route, when the dose of antigen is sufficiently large.

These experiments, which supplement the corresponding results obtained with the 90-day serum-sensitized and globulin-sensitized guinea-pigs, may be considered to be conclusive in showing the presence of sensitized elements in the livers of these animals, and shock must be initiated, in many cases, by the interaction of antigen and antibody in the liver. If the sensitizing antigen had not affected any cells in the liver, then, owing to the slow circulation of the portal blood through this organ, the susceptibility of the animals to shock would always have been greater when the reacting antigen was injected into the systemic blood, whereas the opposite was actually discovered. The experiments of Falls (1918) cannot be accepted in this connexion. Falls found that the amount of serum required to cause anaphylactic shock was less when injected into the portal blood than into the systemic circulation, but large doses of serum were given to the animals, and some died, not in acute shock,

but 20–30 min. after the reacting dose was administered. Moreover, the amount of serum injected often approached that which caused symptoms or death in the non-sensitized controls.

Globulin-sensitized and serum-sensitized animals given a reacting injection of serum

As will be mentioned in the discussion, the distinction between serum-sensitized and globulin-sensitized animals, especially at 15–20 days, plays an important part in the argument already put forward (Winter, 1944*a*) that serum contains a complex protein. It has been shown that, when they first become sensitized, the serum animals may be desensitized by globulin without shock, and require less of this protein to effect desensitization than is needed by globulin-sensitized guinea-pigs; the latter, moreover, are more susceptible to shock, after an injection of globulin, than are serum animals. It is well known that the sensitization of guinea-pigs to proteins is influenced by many factors, among these being the quantity of protein injected. The protein content of horse serum is variable, but there was probably always more protein in the 0·1 c.c. used for the sensitizing injections than was present in the 2 mg. of globulin, containing some ammonium sulphate, by which the globulin animals were sensitized. Certain earlier observations make it unlikely that the differences in the amounts of protein in the sensitizing injections were sufficiently large to have an appreciable influence on the characteristics which have been found, in serum-sensitized and globulin-sensitized animals, given the reacting injection of globulin directly into the circulation.

It was shown (Winter, 1944*a*, Tables 19, 20) that when serum-sensitized and globulin-sensitized guinea-pigs were desensitized by serum injected into the duodenum, the former animals required three times more serum than the latter. Similar indications were obtained in experiments recorded in Tables 10 and 11 of the same paper, when the reacting dose of serum was injected into the portal blood; the serum-sensitized animals required more serum for desensitization than did those sensitized by globulin. While the serum-sensitized guinea-pig requires less globulin for desensitization than the globulin animal, the position is reversed when serum is the reacting antigen; this suggests that the characteristics of the serum-sensitized and globulin-sensitized guinea-pigs are determined only by the nature of the protein used in the initial injection, and are independent of small differences in the amount of the antigen. When the experiments in Tables 10 and 11 of the previous paper were performed, only a few were carried out at the minimum level of serum required to desensitize the animals, and no stress was laid upon these, since at that time they seemed to be of no special interest. Owing to the very small quantity of serum required, the difficulty of determining the minimum amount needed for desensitization was pointed out.

In the experiments about to be recorded, the same technique was employed; large animals were used, and all were given a reacting injection of 0·01 c.c. of serum. The same sample of serum (kept at $+3^\circ$ C.) was used for all the reacting injections, and the experiments were completed in 3 days. Animals of uniform size were chosen, and the average weights of the serum-

sensitized and globulin-sensitized series at the time of operation were almost identical, being 414 and 413 g. respectively. Previous findings (Winter, 1944*c*) made it advisable to give the injections into the portal blood in some animals of each type, and in others into the systemic circulation. In one group, the first horn was removed, serum was injected into the portal blood, and each animal was killed 3½ hr. later. In the other group, the first horn was removed and tested for sensitivity; 3 hr. later, the anaesthetic was given, serum was injected into the jugular vein, and the animal was killed 15 min. later. The results are given in Table 5, and show a marked difference between the animals sensitized by the two proteins. As was to be expected (Winter, 1944*c*), the animals used in Table 5 were also differentiated by the occurrence of shock. No symptoms were noted in any serum-sensitized guinea-pig: four of those sensitized by globulin suffered from shock; one died, the others recovered.

TABLE 5. Globulin-sensitized and serum-sensitized animals (18–20 days) given an injection of 0·01 c.c. serum

	Reaction of 2nd horn	
	Globulin animals	Serum animals
Injection into portal blood	− − − −	− + − + +
Injection into jugular vein	− − − + −	+ − + +

DISCUSSION

Comparison of earlier results (Winter, 1944*a*, Tables 4, 5) with Table 3 of the present paper, shows that, in the present experiments, the amount of globulin required to desensitize animals which were sensitized by this protein, was very much smaller. Denaturation of the globulin used in the first experiments must have occurred to a considerable extent during the preparation. I am at a loss to account for such a pronounced variation in activity, when the same process of isolation was followed. The responsible factor is not likely to be the grinding of the dry protein, since the albumin used in the present work was also more active than that previously prepared (cf. Table 1 of this, with Table 3 of the original paper), and the albumin requires no grinding in the true sense, the crystalline mass breaking up almost at a touch. It is possible that the antigenic properties of proteins are always impaired to some extent by isolation, and it is clearly essential that when comparison is made between the amount of protein which is required to desensitize different animals, the same sample of antigen should be used, a procedure which has been followed in the present work. When a new preparation is made, this must be tested for activity against previous samples.

In the first paper (Winter, 1944*a*), one sample of globulin was used for the experiments in Tables 1–7 inclusive; a second for Tables 8–24, and a third for the remainder of the experiments in the first, and all involving the use of globulin in the second paper (Winter, 1944*c*). One sample

of albumin was used for the experiments of Tables 2–22. Two more preparations of albumin were then made. One was used for the remaining injection experiments of the first, and for those of the second paper, but both were used at different times for the tests on isolated uteri of serum-sensitized and albumin-sensitized animals reported in the last part of the first paper (1944*a*).

The use of different protein preparations has diminished the difference between the amounts of globulin required to desensitize serum-sensitized and globulin-sensitized guinea-pigs, but this difference is still marked, as may be seen by reference to Tables 2 and 3 of the present work. Two other series of experiments in the original paper need to be repeated, for the same reason. The first, showing the effect of albumin in enhancing the desensitizing power of globulin on the globulin-sensitized animal (Winter, 1944*a*, Table 14), has already been repeated, using the same preparations of globulin and albumin as for other experiments in the present paper. Eight experiments were performed, and the amount of globulin needed was found to be reduced to one-thirtieth of that required when globulin alone was injected. The second series, whose validity is in doubt, is that in which animals were sensitized by globulin, followed at 3 weeks' interval by albumin (G–A animals); it is my intention to repeat this series at the first opportunity.

Until it can be said with certainty that no significant impairment of the antigenic properties of the albumin and globulin has occurred during the process of isolation, the evidence that the desensitizing action of serum is due to a protein complex, and not to free globulin and free albumin, must rest on the action of serum when absorbed from the intestine. Attention has been drawn to this question in the first paper, in which it was stated: 'Appreciable denaturation would reduce the value of conclusions drawn from the difference found between serum and the proteins derived from it, when injected into the circulation. On the other hand, denaturation would enhance this difference when the intestinal route was being used; under these conditions serum was shown to be much less active than albumin and globulin.'

That the sensitizing action of serum is due to a complex protein is probable from the evidence which has already been presented for the influence of the 'masked' albumin in the isolated uteri of animals which have been sensitized by serum for 28 days or longer. There was no sensitiveness to albumin in the uteri of the 28-day serum-sensitized animals in these experiments; like those of globulin-sensitized guinea-pigs, the uteri responded strongly to globulin and were qualitatively identical. The essence of the problem is whether, if free albumin is present in serum, the albumin is antigenically inert and takes no part in the sensitization, in which case serum-sensitized and globulin-sensitized animals should be identical; or whether the albumin influences the globulin so that serum-sensitized animals differ from those sensitized only by globulin, although no difference can be shown by the response of the

isolated muscle. It is highly probable, from the evidence presented in this and earlier papers, that serum-sensitized and globulin-sensitized guinea-pigs are not identical. The reason for the discrepancy between these results and those of Dale & Hartley (1916) has not yet been found, but it is important to note, by reference to Tables 2, 3 and 5, that serum-sensitized and globulin-sensitized animals differ, both in their sensitiveness to the reacting protein, and in the amount of this protein required to desensitize the tissues, when they have been sensitized less than 21 days, i.e. at a time when all observers are agreed that sensitiveness to albumin cannot appear in the uterus. It is difficult to explain these differences otherwise than as indicating that the albumin is combined with the globulin of serum to form a complex protein.

The changes in the sensitization mechanism in the guinea-pig which have been found to occur as the time of sensitization increases, present a problem of unusual complexity. In the three types of animal studied, there was in two, viz. in the serum and in the globulin animals, an increase in the sensitivity to the reacting antigen at 50 days, shown by the reduced amount of protein necessary to cause anaphylactic shock. At 90 days, there was a further increase in sensitivity, with the possible exception of the serum-sensitized guinea-pig. As regards sensitivity, therefore, the results were generally consistent in the three groups. The changes in the amount of antigen, however, which was required to desensitize the animals, showed no such consistency. In the serum-sensitized guinea-pigs, the amount of globulin which was needed to abolish the reaction of the uterus increased at each period; in the globulin-sensitized and albumin-sensitized animals, the amount of antigen which was required to desensitize the tissues appeared to vary independently of the amount which was necessary to cause shock. It has already been remarked (Winter, 1944*a*) that the reaction of the sensitized uterus is of little use as a guide to the response of the whole animal, when the reacting dose of antigen is injected, since some animals may respond by shock, while others, of apparently equal uterine sensitivity, show no reaction. It is clear that, after the injection of the reacting dose of protein into the sensitized animal, fixation of antigen may occur in all, but causation of shock only in some cases. Probably two factors are involved; it is the shock-producing factor alone which has been extensively studied. In guinea-pigs which have been sensitized only for a few weeks, the shock-producing mechanism is but slightly developed; at this time many animals may be given the reacting injection without shock; in the case of the serum-sensitized guinea-pig, given globulin, no animal has shown symptoms. After some months, many animals suffer from shock, but even at this time, some may be desensitized without any reaction.

From the results obtained in the present work, we may conclude that, in animals sensitized for some months, when sufficient antigen is injected to

desensitize the uterus, only a very small fraction is needed to set in motion the shock-producing mechanism. This mechanism is believed to be the release of preformed toxic substances from sensitized cells in the animal, following the antigen-antibody reaction (for a recent review, see Dragstedt, 1941). Schild (1937) has shown that the anaphylaxis reaction in the guinea-pig leads to the release of histamine-like substances from many tissues. The means by which toxic substances are released by the antigen-antibody reaction is still unknown, and it may well be that the release of these substances in amount necessary to cause symptoms does not occur in every animal, nor always from the same tissue. It is remarkable that the greatest release of histamine, in Schild's experiments, occurred in the aorta, while the liver was inactive.

It is difficult to accept the view (Dragstedt, 1941) that the toxic substances released by the antigen in anaphylactic shock in the guinea-pig are 'normally resident' in various tissue cells, without some qualification. Many more guinea-pigs which have been sensitized for some months suffer from anaphylactic shock, than those which have been sensitized only for a few weeks. Two possibilities must therefore be borne in mind. It may be assumed that, in the shock-producing mechanism, the first event is cell damage induced by the reaction between antigen and antibody, and this allows the release of toxic substances into the blood. The toxic substances, if present in the cells in the same amount in both types of animal, must be released with greater speed, after more extensive cell damage, in the guinea-pigs which have been sensitized for 90 days; the rate of release in the 15–28-day animals must be, in many cases, too small for the toxic substances to cause symptoms. If the rate of release in the two types of animal is approximately equal, on account of a similar extent of cell damage, the toxic substances must be present in greater amount in the cells of the 90-day guinea-pig, and one must therefore conclude that the sensitizing antigen increases the formation of these substances in the cells, as the duration of sensitization advances.

Summary

1. Guinea-pigs have been sensitized by horse serum, albumin, and globulin respectively. A study has been made at different intervals after sensitization, of the susceptibility of the animals to shock, and of the amount of antigen required to desensitize the tissues *in vivo*. Only the changes in susceptibility to shock were consistent in the three types of animal.

2. Further evidence is adduced which indicates that serum contains a complex protein, and it is shown that the characteristics of serum-sensitized and globulin-sensitized guinea-pigs are independent of small differences in the amount of protein in the sensitizing antigens.

3. When a very small amount of protein is given in the reacting injection to highly sensitized animals, shock is more frequently caused when the in-

jection is made into the portal blood, as compared with injection into the systemic circulation. It is concluded that, in the three types of animal studied, sensitization of cells occurs in the liver.

4. It has been found that albumin and globulin, when prepared from horse serum by the same method, may vary in antigenic activity. The bearing of this finding on the author's previous results is discussed.

For the supply of animals I am indebted to Dr S. W. F. Underhill and the Director of the Agricultural Research Council Field Station; to the latter I am also indebted for keeping some animals until they attained specified weights. I wish to thank Dr A. McDiarmid for giving some of the sensitizing injections. In this laboratory, Messrs R. Bradey and G. Webber were in charge of the animals; for the care they have taken and for much technical assistance I express my gratitude. My thanks are due to the Government Grants Committee of the Royal Society for defraying the heavy expense of purchase and maintenance of the animals.

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THE ELECTRIC RESPONSE OF THE HUMAN EYE

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The present work arose from an investigation of the electrical changes in the human brain, particularly those produced by the exposure of the eyes to a flickering light. Some years ago Adrian & Matthews (1934) found that potential waves, in phase with the flicker, could be recorded from the occipital part of the head. As a rule, the frequency of the occipital waves agreed with that of the flicker, but sometimes it occurred at double the rate and, as this doubling complicated the analysis of the cerebral events, it seemed necessary to find out how it arose and, in particular, whether it arose in the brain or in the eye. The simplest method of attack was to try to record the potential waves generated in the retina as well as those in the brain. The attempt has been successful to the extent of showing that a double response may certainly arise in the eye, but the more important finding is that the human electroretinogram can be recorded without much difficulty and can give fresh information about the retinal mechanism.

What follows is mainly concerned with the potential changes occurring in the eye in response to single flashes of light. Responses to flickering light are described in a later section. The doubling of the cerebral flicker rhythm will be dealt with elsewhere, although, in fact, it was the starting-point of the investigation.

Previous work on the human electroretinogram is summarized in a paper by Cooper, Creed & Granit (1933). It has been established that the response of the human eye has the same general form as that recorded from other vertebrate eyes, isolated or *in situ*, or from the exposed retina, but technical difficulties have prevented any further study of it. One of the chief difficulties has been that eye movements are hard to avoid and give rise to potential changes resembling those of the electroretinogram. In the present experiments they have not caused much trouble, partly no doubt because the subject learnt to control them, but mainly because of the shortness of the period of illumination. As a rule the stimulus was a flash lasting not more than 1/20 sec., and no serious attempt was made to record with exposures lasting more than a second. Thus the inconveniences of a wandering base-line have not been serious.

Method

Electrical recording. A simple electrode system and a standard recording instrument have been used from the beginning. The electrodes are (1) a clip fastened to the lower part of the cheek, and (2) a moist thread making contact with the exposed part of the eye-ball on the nasal side of the cornea. The thread protrudes 2–3 mm. from a narrow glass tube filled with saline and fixed with plasticene between the bridge and the lens rim of a pair of spectacles. One end of a fine silver wire is coiled round the thread near its exit from the tube; the other end connects with an insulated lead fastened to the side member of the spectacle frame. The coil of silver wire in the tube is coated electrolytically with silver chloride. To protect it from the light the tube has sometimes been shielded by a wrapping of black paper or a coat of black paint. This precaution has not always been taken and is unnecessary except with very strong illumination, for the potentials arising in an unshielded electrode on exposure to a flash of light are very much smaller than those produced by the eye.

The electrode is fixed so that when the spectacle frame is in its proper place the moist thread touches the conjunctiva somewhere between the margin of the cornea and the inner canthus of the eye. There is no discomfort if care is taken not to move the eye after the contact is made and there has been no need to use local anaesthetics. Between each set of recordings the electrode is moved away from the eye, since the desire to blink becomes imperative. When it is replaced there is no guarantee that the thread will touch exactly the same point on the eye as before, but it is found that this makes very little difference to the size of the potential waves (cf. Fig. 2B).

The chief drawback with this method of leading from the eye is that eye movements, and especially blinking, disarrange the electrode. Consequently, the records must all be made in less than 2 or 3 min. after the electrode has been adjusted. The adjustment has to be done in the light before a mirror, and, though a red light can be used, there is bound to be some interference with the progress of dark-adaptation. For exploratory work this does not matter very much, but it would be troublesome if exact measurements had to be made of the rate of dark-adaptation.

The potential changes were recorded by a Grass three-channel ink-writing oscillograph of standard pattern. This has amplifiers with resistance-capacity coupling which can be set so that, if a steady potential difference is applied, the deflexion falls to half its initial value in 0·1 or 0·4 sec. Even with the longer coupling the instrument could only be used for recording the response to a relatively brief exposure, but changes occurring in the first $\frac{1}{10}$ sec. will be more or less faithfully reproduced. Since it gave a steadier base-line it was often more convenient to use the shorter coupling for preliminary observations, checking them afterwards with the longer. An ink-writing oscillograph is, of course, unable to follow extremely rapid changes of potential, but the electroretinogram is slow enough to be within its range. Indeed, for the short exposures used in these experiments, it is doubtful whether an instrument with direct-coupled amplifiers and photographic recording would have revealed more, and certain that the difficulties of the experiments would have been much more formidable.

Optical system. In the earliest experiments the source of light was a 75 W., 12 V. headlight bulb, in series with a variable resistance and connected with the 100 V. mains, so that it could be overloaded if necessary. Later, the range of illumination was extended by using either a 1000 c.p. 'Pointolite' lamp or an arc lamp, the intensity being controlled by neutral filters. The light was collected by a condenser, passed through a cooling trough and brought to a focus in the plane of a large wooden disk rotated at a constant speed by a gramophone motor, and having a slit which could be varied in width. A shutter in front of the disk could be raised and lowered so as to give a single flash or a series. Beyond the rotating disk the beam passed through a second condensing lens to illuminate the screen which was viewed by the subject. A small part of the beam was deflected on to a photoelectric cell leading to one channel of the oscillograph, so that the duration and intensity of the flash could be recorded, as well as the electric response of the eye.

Wratten and Ilford colour filters were used for producing approximately monochromatic light. The optical properties of these filters are well known and they have often been used in similar experiments (cf. Graham & Hartline, 1935). Though they are not strictly monochromatic, only a narrow band of wave-lengths is passed by each filter, and the energy value of the light does not vary too greatly from one to another. The transmission of the different filters is shown in Fig. 1 in relation to the scotopic and photopic luminosity curves of the eye.

Two arrangements of viewing screen were used. One was a large white surface, 4 ft. square. On this the light could be thrown in a disk up to 2 ft. in diameter, and the size and shape of the field could be varied by suitable diaphragms and stops. The screen was fixed to the wall of a small dark room, the light was admitted through an opening in the opposite wall, and the subject sat facing the screen with his chin supported in a rest 2 ft. away from it. The other arrangement

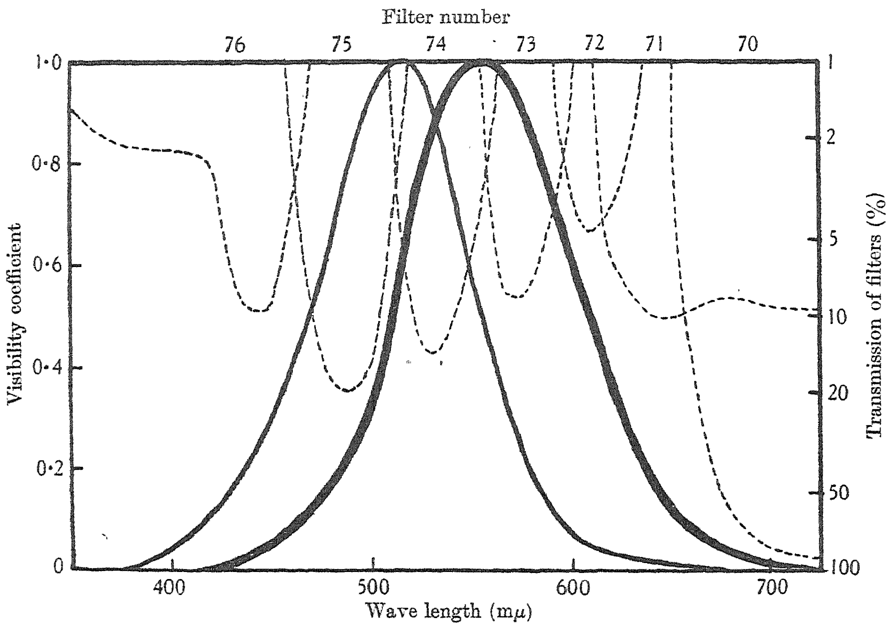

Fig. 1. Wave-lengths transmitted by Wratten colour filters, nos. 70–76, compared with the scotopic and photopic visibility curves of the human eye.

was that originally devised for work on the flicker potentials of the brain where a large uniform field is needed. An opal glass bowl 11 in. in diameter was fixed in the opening in the wall of the dark room with its convex side facing the beam of light. The subject sat inside the room with his head in the concavity of the bowl, his chin resting on its rim. When the whole of the bowl was illuminated, only the extreme periphery of the visual field was in obscurity, and over most of the field the brightness was even. As the bright surface was very close to the eyes, fixation was impossible and attempted fixation movements did not intervene to spoil the record. This was the more convenient arrangement for all experiments in which the size of the field was not to be varied, but the results were substantially the same whichever screen was used. With the bowl, the brightness is given in equivalent foot candles (e.ft.c.) obtained by dividing the illumination on the convex surface by the transmission factor of the opal glass.

Experimental procedure. In most of the work the experimenter was his own subject, operating the oscillograph motor and the light shutter by strings led into the dark room. Between each series of recordings the light was switched on, and the subject disconnected the leads and went

to the outer room to inspect the records, alter the light, etc. If the routine was as regular as possible, the eye could be brought to a fairly steady degree of dark-adaptation, so that, for a given flash after a given period in complete darkness, the responses would be reasonably constant. As the rooms were never brightly lit the normal state of the eye was one of moderate dark-adaptation. Light-adaptation was produced by viewing a large opal screen lit from behind by a 200 W. lamp or by going outside the laboratory and looking at the bright sky. With the latter method at least half a minute had to elapse before a record could be taken. Records have also been made with the screen continuously illuminated, the flash increasing the brightness momentarily.

Results

1. *The response to single flashes*

Nature of records. The accuracy of recording with the foregoing technique may be judged from Fig. 2. In Fig. 2A the eye was exposed to a flash of white light lasting 1/40 sec. with a brightness of the screen of 32 e.ft.c. The response is a diphasic potential change with the conjunctival lead becoming

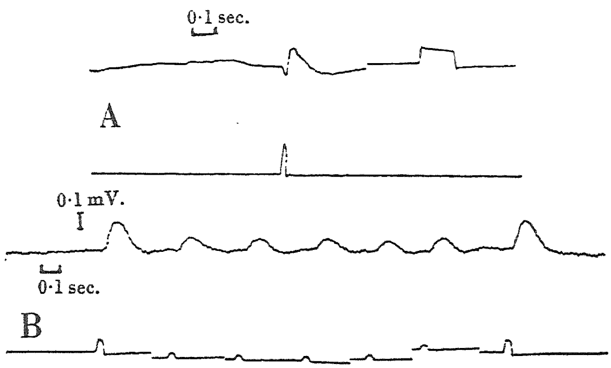

Fig. 2. A. Response to flash of white light followed by calibration curve (0·1 mV.). The photoelectric cell record below signals the duration and intensity of the flash, in this case 0·025 sec. with a brightness of the screen of 32 e.ft.c. B. To show consistency of recording. Blue light (Wratten screen 76). The conjunctival electrode was removed and replaced after each record. Brightness 1·2 e.ft.c. for first and last response, 0·24 e.ft.c. for the five smaller responses.

first negative and then positive to that on the cheek. The maximum change of potential is 0·12 mV. Below the record from the eye is that from the photoelectric cell illuminated by the same flash. The calibration curve which follows shows the degree of accuracy to be expected from the record. The sensitivity is that usually employed, for the greatest potential changes encountered are of the order of 0·3–0·4 mV., and the unsteadiness of the base-line (probably due to slight eye movements) prevents the recording of potential changes less than 0·01 mV.

Fig. 2B shows that the potential changes stay reasonably constant in spite of readjustment of the electrodes. In this case, blue light was used, and the intensity of the flash was chosen to give responses of about two-thirds or

one-third of the maximum potential obtainable with the particular arrangement in use. Between each response the conjunctival electrode was removed and replaced, the record being made after 20 sec. in darkness. Records made on successive days show just as little variation, provided that the degree of dark-adaptation is the same. To secure this a few small holes and cracks in the chamber wall were used as sources of very feeble illumination which would become visible when the standard degree of dark-adaptation was reached.

Photopic and scotopic responses. The principal result has been the demonstration that the electric response to a flash may show two distinct components, one of which seems to be mainly due to the photopic mechanism and the other to the scotopic. The appearance of these two components is illustrated in Fig. 3, which gives the response to a brief flash (*a*) of deep red light which should stimulate mainly the photopic system, (*b*) of blue light

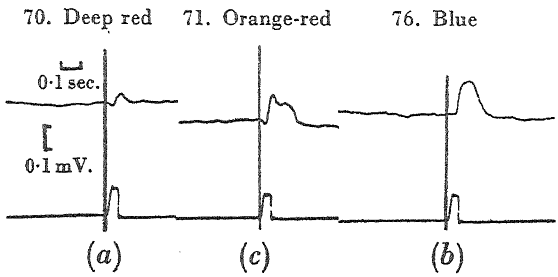

Fig. 3. Records showing the dual character of the response. Brief diphasic response with red light (screen 70, 5 e.ft.c.), longer monophasic response with blue (screen 76, 7 e.ft.c.) and composite with orange-red (screen 71, 11 e.ft.c.).

which should stimulate mainly the scotopic, and (*c*) of orange-red light which should stimulate both, but the photopic more than the scotopic. It will be seen that in (*a*) the response is a brief diphasic potential change lasting about 0·1 sec., with the conjunctival electrode becoming initially negative, in (*b*) it is a monophasic change, positive in sign and lasting at least twice as long, and in (*c*) it is a complex change which could be produced by adding the brief and the slow responses together.

Fig. 4 summarizes the chief evidence for regarding the brief response as characteristic of the photopic mechanism and the longer monophasic response as characteristic of the scotopic. Fig. 4A shows the responses of the light-adapted eye to brief flashes of various wave-lengths. In the fully light-adapted eye the threshold of the scotopic receptors would be greatly raised, whilst that of the photopic would be much less affected, so that we should expect that the response would be mainly that of the photopic mechanism. It is, in fact, for all colours except deep blue, the same kind of brief change as that shown in Fig. 3 (*a*). With the particular brightness employed the response

to blue is so small that its form cannot be made out. This, too, is to be expected, since blue light would not stimulate the photopic receptors, and the threshold of the scotopic has been raised by the light adaptation so that in fact only a very slight visual sensation is produced.

Fig. 4B shows the effect of similar flashes when the eye is moderately dark-adapted. The responses of the scotopic mechanism should now be in evidence with every colour except deep red, which does not stimulate it, and the response of the photopic mechanism should be present also with every

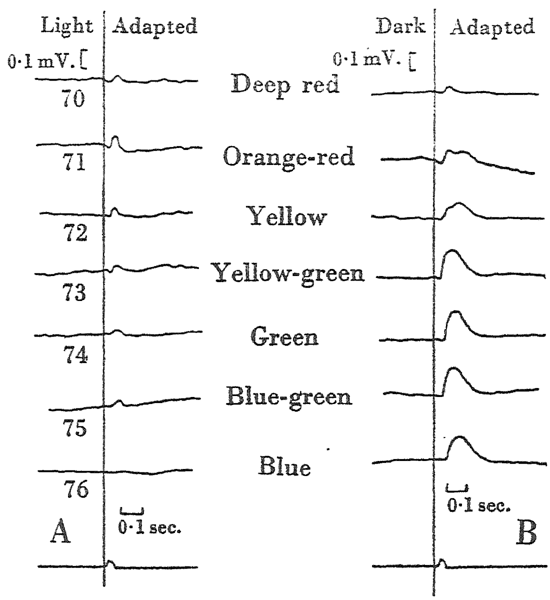

Fig. 4. Response to various wave-lengths in (A) light-adapted and (B) dark-adapted eye. The slower component is only present in B. The light source has the same intensity throughout but the brightness of the screen varies with the colour filter in use.

colour except blue. It will be seen that the responses to red and blue have the contrasting forms already described, whilst the responses to intermediate wave-lengths seem to be compounded of both types. In the response to orange-red the slow and rapid positive waves are easily separated; with yellow, yellow-green and blue-green the slower positive wave is larger and is merged more or less with the rapid wave.

Changes of intensity. These records are enough to establish a *prima facie* case for regarding the two components as due to the photopic and scotopic mechanisms respectively, but there are several points which need closer examination. In Fig. 4 the intensity of the light falling on the colour filters was the same throughout, but as the transmission factor is not alike for all the filters the energy value of the different flashes of coloured light was not

the same. It could have been made so easily enough, but what is really needed is an answer to the question whether the appearances shown in Fig. 4 are to be found only over a narrow and selected range of intensities, or over most of the range to which the eye is normally exposed.

It is not easy to produce a very bright flash of monochromatic light over a large field, yet, if the light is dim, the field must be large if it is to produce a measurable potential change. However, the intensity of the light can be readily increased if the field is made smaller, and, with a bright light, a measurable potential change can still be produced. A reduction in the area of the retina stimulated by a red or a blue flash has no effect on the form of the potential change though it naturally reduces its magnitude (if the intensity of the light is not altered). Thus we can secure a reasonably wide range of stimuli by using a large field for the dimmer light and a much smaller field lit by a concentrated beam when we need the highest intensity.

With the largest possible field (the concave opal bowl) and an exposure of 1/30 sec. the minimum intensity of blue light (Wratten screen 76) required to give a measurable response has been 0·003–0·004 e.ft.c. This value could no doubt be lowered by more prolonged dark-adaptation, but it must be well above the visibility threshold, since the potential change must be large enough to stand out against the base-line fluctuations. With deep red light (screen 70) the minimum intensity needed for a response is much higher (about 0·5 e.ft.c.), partly, no doubt, because the photopic mechanism sets up smaller potential changes, but mainly because the visibility threshold is also much higher.

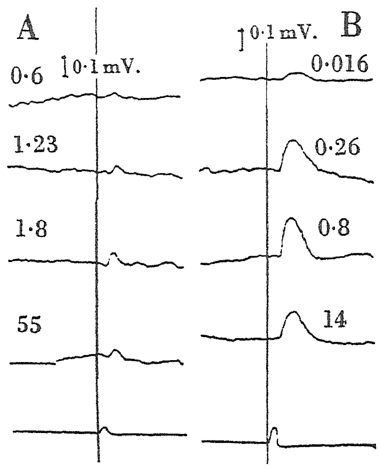

Fig. 5. The form of the response to red and blue is not affected by changes of intensity. A. Response to red (screen 70) with brightness of 0·6, 1·23, 1·8 and 55 e.ft.c. Field reduced in last record. B. Response to blue (screen 76) with brightness of 0·016, 0·26, 0·8 and 14 e.ft.c. Field reduced in last record.

With the largest field, the intensity could not be raised to more than 1·2 e.ft.c. for blue light or 3·7 for red, but with a field reduced to 2 cm. diameter the brightness could be raised to 15 e.ft.c. for blue and 70 for red. Records made at various intensities within this range are given in Fig. 5. They show that the characteristic form of the response to red is quite unaffected by a 100-fold increase in intensity and that to blue by a 900-fold increase. Other experiments have given the same result with a 1–4000 fold increase in the blue light.

With the strongest red light and the eye dark-adapted, there is sometimes a small slower component following the initial diphasic effect, and with strong blue light there is sometimes an initial negative swing, though it is scarcely larger than the random fluctuations in the base-line. It is not surprising that strong red light should be able to affect the scotopic receptors or strong blue light the photopic, yet it can be said that the distinctive form is preserved over a fairly wide range of stimulus intensities and a corresponding range in the size of the response. With light of intermediate wave-length the form of the response ought to change with increasing brightness if it is produced by two components of different threshold. That it does so is shown in Fig. 6

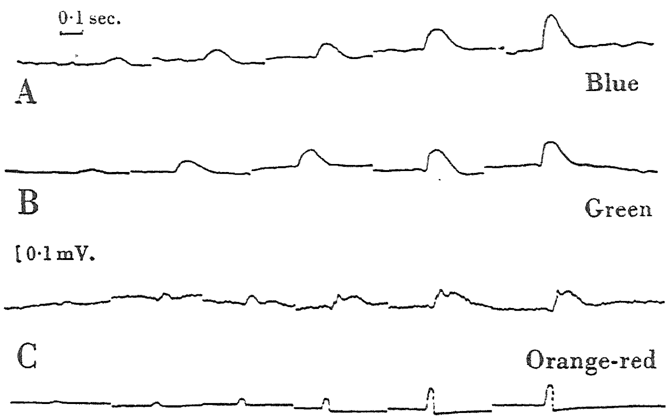

Fig. 6. The form of the response to intermediate wave-lengths varies with the intensity. A. Responses to blue (screen 76), showing no change in form. Brightness 0·03, 0·20, 0·28, 0·48, 1·1 e.ft.c. B. Responses to green (screen 74). The photopic component appears at higher intensities. Brightness 0·015, 0·08, 0·22, 0·54, 1·1 e.ft.c. C. Responses to orange-red (screen 71). The scotopic component appears at higher intensities. Brightness 0·78, 1·6, 3·12, 7·8, 15·6 e.ft.c.

which gives responses to blue, green and orange-red. A feeble green light gives no sign of the initial rapid effect, but it becomes more and more prominent as the intensity is raised. With orange-red light it is the slower component which is absent at low intensities. It should be emphasized that it is the short duration of the positive wave which is the main distinguishing feature of the photopic component. The initial negativity may be very small, and though it only appears when the stimulus would be likely to affect the photopic mechanism there is reason to believe that it may be due to a process not directly concerned with the excitation of the receptors (cf. p. 97).

Effect of dark-adaptation. Another point to be considered is the extent to which the prominence of the longer 'scotopic' response can be correlated with the degree of dark-adaptation. It has been shown that it appears when the eye is dark-adapted, but if it is really the response of the scotopic

mechanism we should expect that its rate of increase would run parallel to the increasing sensitivity of the eye. If it appeared abruptly at a certain stage of dark-adaptation or reached its maximum long before dark-adaptation was complete, it would be more difficult to accept it as the scotopic response.

Owing to the need for some illumination of the eye when the electrode is adjusted, it is impossible to record the response immediately after a long period in complete darkness; but records may be taken after 2 or 3 min., and the red light, by which the adjustment is carried out, need not be bright enough to interfere much with the general progress of dark-adaptation. The development of the slow response certainly runs parallel to that of dark-adaptation in that the increase begins at once and continues for half an hour or longer. This is illustrated in Fig. 7. In Fig. 7A, for instance, the response to blue light begins to increase in the first 2 min., and it is still increasing,

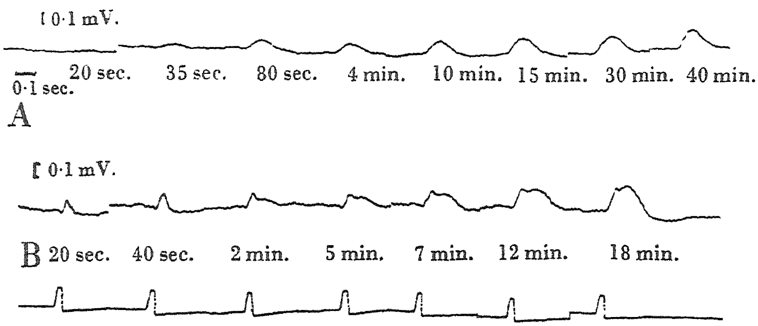

Fig. 7. Progressive increase in slow component with dark-adaptation. A. Responses to blue (screen 76) at various times after beginning of dark-adaptation. Brightness 0·09 e.ft.c. B. Responses to orange-red (screen 71). Brightness 5·2 e.ft.c.

though much more slowly, half an hour later. In Fig. 7B, with orange-red light, the slow component of the dual response increases progressively in the same way. To obtain accurate figures for the whole course of dark-adaptation would involve a change of technique. In spite of this, we can be fairly sure that the increase in the response follows the same general time course as the increase in sensitivity of the eye, for it is found that at all stages of dark-adaptation one can confidently expect to find an electric response just large enough to detect, whenever the sensation produced by the flash exceeds a certain small luminosity.

Changes in duration of flash. Since the eye (in animals) and the optic nerve both respond to darkening as well as to illumination, it is worth considering whether the later part of the human electroretinogram in records such as those in Fig. 7B might be partly or wholly an 'off effect', instead of the response of the scotopic mechanism. This would leave unexplained the difference in the responses to red and blue light, but there are two more

arguments against it. One is that the slower component of the response still appears though the duration of the flash is reduced to 1/100 sec. (Fig. 8A). The other, and more conclusive, is that the slower component appears in its usual position although the flash is prolonged for half a second or more (Fig. 8B). Indeed, with a flash of long duration, it is possible to make out a small 'off effect' at the end of the electroretinogram, separate from the positive wave nearer the beginning.

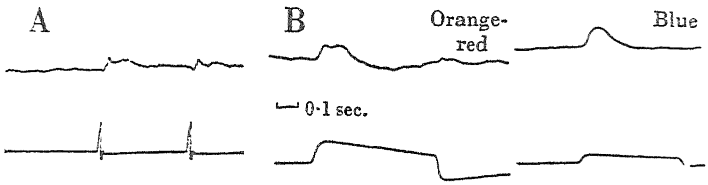

Fig. 8. Records showing that the slower component is not an 'off' response. A. Orange-red flash lasting 0·01 sec., 15·5 e.ft.c. B. Orange-red and blue flashes lasting 0·5 sec., 7·8 and 0·9 e.ft.c.

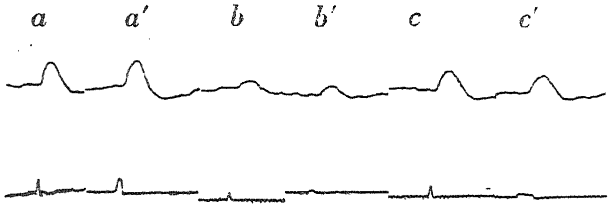

Fig. 9. Parallel effect of changes in intensity and duration. Blue light (screen 76):

	Brightness (e.ft.c.)	Duration (sec.)	Product
a	0·6	0·007	42
a'	0·2	0·028	56
b	0·2	0·007	14
b'	0·04	0·028	11
c	0·2	0·014	28
c'	0·04	0·084	33

When the duration of the flash is short, the size of the response, like the resulting sensation, is determined by the product of the intensity and the duration of the light, i.e. by the quantity reaching the eye. Fig. 9 gives a series of records illustrating this, and Fig. 10 shows the relation between the intensity and duration required for responses of equal size with blue light. The response ceases to depend on the product of intensity and duration when the flash is longer than about 1/15 sec. for blue light and 1/20 sec. for red. A reduction in intensity cannot then be compensated for by a further increase in the length of the flash, for it is the duration of the response and not its size which is increased. It is noteworthy that, when the flash lasts long enough to cause this lengthening in the retinal response, there is also a marked

change in the sensation, which now seems to last an appreciable time. The critical duration is certainly shorter for red light than for blue, but there is a considerable margin of error in determining it.

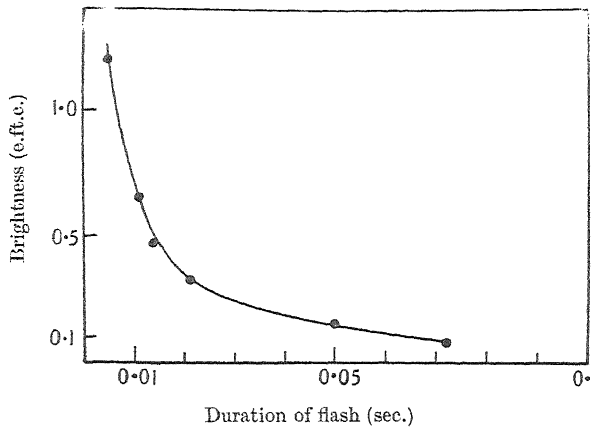

Fig. 10. Relation between intensity and duration of flashes giving a response of fixed size. Blue light (screen 76).

Localization. Since the photopic receptors are more concentrated in the centre of the retina and the scotopic at the periphery, we might expect that the brief response would be more dependent on the brightness of the central part of the visual field and the slower component on that of the periphery. It is not possible to obtain a measurable response when the illumination is restricted to the rod-free area of the retina, but we can use a large field divided into central and peripheral parts and compare the responses due to either of them. The arrangement finally adopted was one in which the light fell in a disk 2 ft. 6 in. diameter on an opaque white screen 2 ft. from the eye. Diaphragms were arranged so that the illumination could be confined to a central disk 6 in. in diameter or to the annulus round it. With blue light the response due to the whole field was not much larger than that due to the peripheral annulus and the response to the central disk was much smaller (Fig. 11 A). With red light, on the other hand, the responses due to the annulus and to the central area were nearly equal (Fig. 11 B).

This indicates that the receptors sensitive to blue and giving the slower monophasic response are less concentrated at the centre than those sensitive to red and giving the shorter diphasic response. To complete the evidence, however, the same sort of difference must be established for the two components of the response to an intermediate wave-length which would stimulate both photopic and scotopic receptors. Results with orange-red are given in Fig. 12. The contrasting effects of the central and peripheral fields on the two components are not as striking as they are on the responses to red and

blue, yet there is certainly some favouring of the initial diphasic effect by central vision and of the slower monophasic by peripheral.

From one point of view it is unfortunate that the retina cannot be regarded as a surface of uniform sensitivity and structure, for, if it were so, the relation between the potential developed at the electrode and the distance and size of the stimulated area would have more significance. In all probability the lead on the eyeball is so far forward that there are no great differences in the resistances between it and different parts of the retina. One would expect that larger poten-

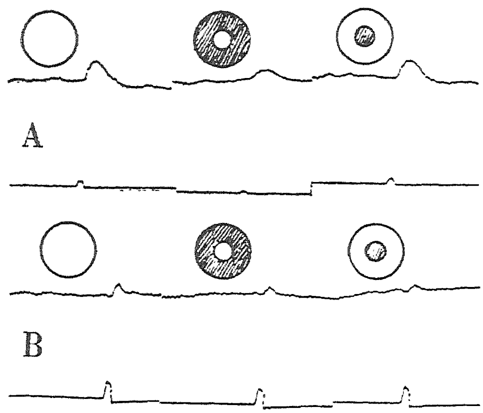

Fig. 11. Effect of restricting illumination to the central or peripheral part of the field A, blue light (screen 76); B, red (screen 70).

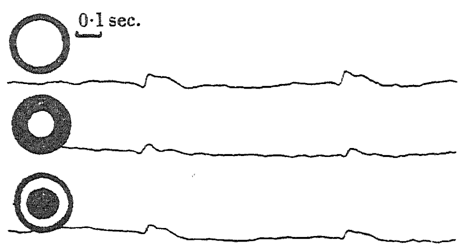

Fig. 12. Effect of restriction to central or peripheral field with orange-red light (screen 71, 14 e.ft.c.). The slow component is relatively smaller when the peripheral field is not lit.

tials would be developed by illuminating a peripheral area on the nasal side of the eyeball (near the electrode) than by illuminating a similar area on the temporal side. Actually the potential is larger, but by not more than 50%. This result may be due in part to stray light affecting other parts of the retina than those directly illuminated. Attempts to lead from nearer the retina in the hope that finer details of the electroretinogram could be studied (cf. Fry & Bartley, 1934) have been so far unsuccessful.

The response to white light. With white light the stimulating effect of a flash can be made far greater than with coloured light, and at high intensities the photopic type of response becomes much more prominent. At very low intensities the response seems to be a pure monophasic effect, a brighter flash

gives the primary negativity and the more abrupt rise of the positive wave and finally at very high intensities the initial negativity may become almost as large as the succeeding positive wave (Fig. 13).

In a preliminary account (Adrian, 1944) it was stated that at high intensities the slower part of the response was definitely reduced, as though there were an inhibition of the rod by the cone mechanism. But further work has

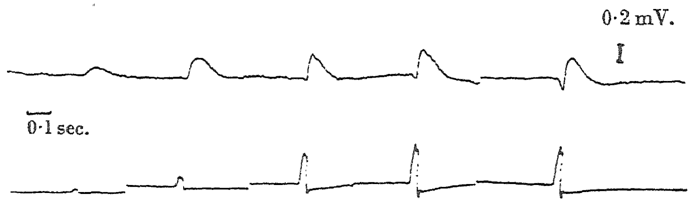

Fig. 13. Response to flashes of white light of increasing intensity. Brightness 0·1, 1·0, 10, 20, 103 e.ft.c. The responses are not strictly comparable, as the degree of dark-adaptation is not constant.

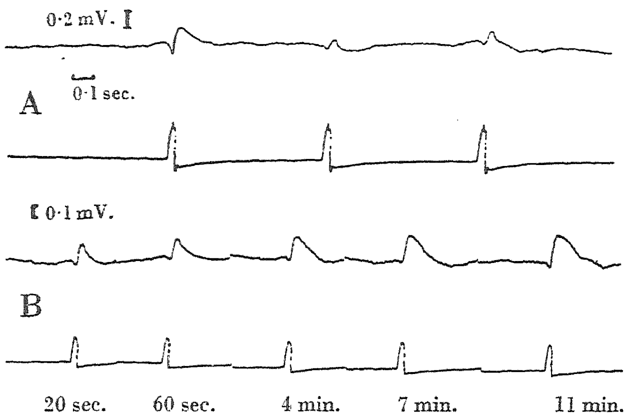

Fig. 14. A. Response of moderately dark-adapted eye to flashes of 103 e.ft.c., showing reduction after the first flash. B. Response at various times after the beginning of dark-adaptation. Flash, 13·5 e.ft.c.

shown that this reduction was due, mainly at any rate, to unsuspected light-adaptation. A single bright flash may reduce the degree of dark-adaptation for several minutes, and it is therefore essential to expose the eye as little as possible and to allow long periods in darkness between successive flashes. When this is done the response does not show an appreciable curtailment of the positive phase, but if the flashes are repeated at intervals of 10 sec. or less the later part of the response soon disappears as dark-adaptation is lost. Even with all precautions to maintain the dark-adapted state, the maximum potential developed in the positive swing is not as large as one might expect. This alone, however, is not enough to prove an inhibitory effect, for it might have many other explanations.

The effect of light- and dark-adaptation can be seen in Fig. 14. In Fig. 14A the moderately dark-adapted eye was exposed to a series of bright flashes at intervals of 1 sec., and at the second flash the slower part of the positive wave has disappeared. The initial negativity is also reduced, but only by about 50%. In Fig. 14B the eye was first light-adapted and was then tested by a flash after various periods in darkness. The initial negativity shows little alteration, but the positive wave is still increasing after 11 min. These records were made with the pupil dilated with 'Paredrine' to avoid the changes which might result from alterations in the amount of light entering the eye.

The relation between the intensity of the flash and the magnitude of the response in the light-adapted eye is not easy to formulate. Judged by the size of the initial negative deflexion there is no sign that the response is near its maximum even with the brightest light possible, but the positive deflexion does not share the same continued increase. In this respect, therefore, the two phases seem to behave differently. In dark-adaptation, too, the negative and positive phases may not increase in size at the same rate. The most likely explanation is that they represent two distinct processes in the retina, as they are supposed to do in the analyses of the electroretinogram proposed by Einthoven & Jolly (1908) and by Granit (1933). The initial negative deflexion may be considered part of the photopic response, in that it is absent with feeble illumination and is not much increased by dark-adaptation, but it is known to occur in eyes which have very few cones and so is unlikely to be specially connected with cones rather than rods.

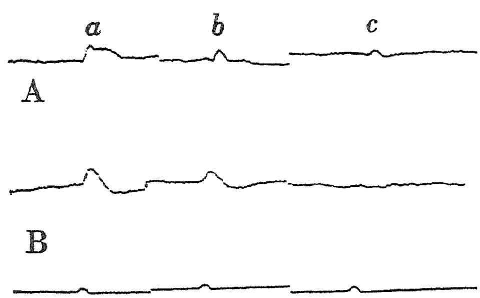

Fig. 15. Response to a flash thrown on a dimly or brightly lit field. A. Orange-red flash of 30 e.ft.c. thrown on field (a) in darkness, (b) lit by 0·3 e.ft.c., and (c) lit by 2 e.ft.c. B. Green flash of 3·5 e.ft.c. on fields as in A.

The response of the eye in light and darkness. In the foregoing experiments the eye has always been in darkness for some seconds before the flash occurs. As the period in darkness would favour the scotopic response, some records were made with the field dimly or brightly lit throughout from another light source. These give some idea of the contribution which would be made by the scotopic mechanism under more normal conditions of vision. Results are

given in Fig. 15 with the field (*a*) in darkness, (*b*) having a brightness of 0·3 e.ft.c., and (*c*) of 2 e.ft.c. With a flash of orange-red light which raises the brightness momentarily to 30–35 e.ft.c., the slow component is present when the field is initially dark but not when it is dimly lit. With green light the slow component appears with the dimly lit field although the flash adds only 3·5 e.ft.c. to the brightness. With the brighter field only the photopic type of response could be recorded; an added flash of blue light could be seen, but it was not possible to use an intensity of illumination great enough to produce a measurable electric response, and white light gave only the brief diphasic effect.

2. *The response of different subjects*

Although most of the information has been derived from one eye (E.D.A., left eye), records have been made from five other subjects with similar results. The responses to red, orange-red and blue in three of them are given in

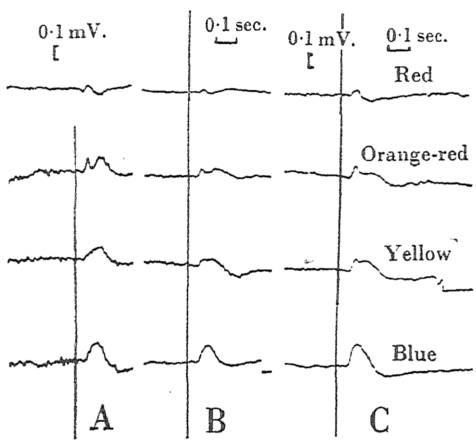

Fig. 16. Responses to red, orange-red, yellow and blue from three different subjects A, B and C, showing composite effect with orange-red and yellow. Records somewhat distorted owing to use of shorter time constant in oscillograph.

Fig. 16. There is the same contrast between the brief response to red and the longer response to blue, whilst orange-red gives the response compounded of both types. Light-adaptation had the same effect, abolishing the slower component of the compound response, but having a relatively small effect on the brief response to red light.

Records were made from one subject who complained of night blindness, but all that could be established was an unusually slow rate of dark-adaptation, judged by the subject's visual sensations and by the progressive increase in the retinal response to blue. Otherwise the scotopic mechanism seemed to

act normally, for the response to blue had the usual duration and reached 0·3 mV. after half an hour in the dark, and the response to orange-red showed both the rapid and slow components.

3. *The response to flicker*

With flashes repeated at intervals of half a second or less the compound nature of the response (except to deep red and deep blue) stands out very clearly, since the two components are very differently affected by an increase in frequency. This applies particularly to the responses at the beginning of a series. The two types of behaviour can be seen from the records in Fig. 17 which were made with flashes of red and blue light repeated at various rates. With red light the successive responses are all alike as long as the frequency is less than about 20/sec.: above this rate the first of the series is larger than

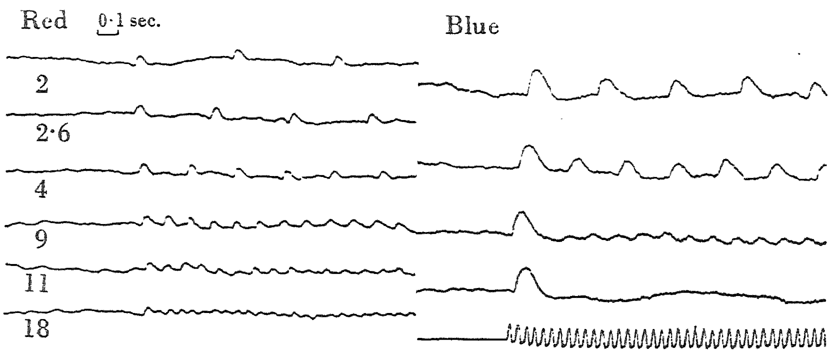

Fig. 17. Response to repeated flashes of red (Ilford screen 609, 1·2 e.ft.c.) and blue light (Wratten screen 76, 0·9 e.ft.c.) at various frequencies, showing reduction after the first response to blue but not to red.

the rest though the difference in size is not very great. With blue light, however, unless the illumination is very low, the frequency has only to be raised to 3/sec. to cause a considerable reduction in the second and later responses, and above 15/sec. all but the first are so reduced as to be only just visible in the record. Now, if for red or blue we substitute white light, or light of some intermediate wave-length, the records obtained could be reproduced by merely adding together the two types shown in Fig. 17. Examples are given in Fig. 18. At frequencies of 10/sec. or more, the first response of each series has a large positive wave, as in the records with blue light, but after this there is only a series of brief spikes like those in the records with red.

The reduction of the scotopic component after the first response is presumably due mainly to light-adaptation. It is not due, except perhaps to a small extent, to contraction of the pupil, for it is equally marked after dila-

tation of the pupil with a mydriatic. It is greater when the stimulating effect of the light is high than when it is low, whether the alteration is produced by changing the intensity of the light or by changing the degree of dark-adaptation of the eye. But, although this suggests a dependence on photochemical factors, the rate of recovery after stimulation might well depend on neural factors also, and its relation to the process of dark-adaptation remains to be worked out.

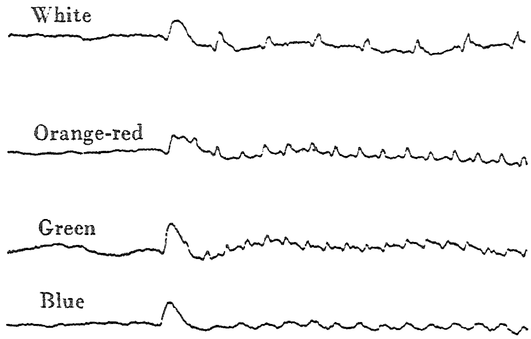

Fig. 18. Response to repeated flashes of white (22 e.ft.c.), orange-red (6·8 e.ft.c.), green (3·1 e.ft.c.) and blue light (1·2 e.ft.c.), eye moderately dark-adapted. The scotopic component of the response is greatly reduced after the first flash. Pupil dilated with 'Paredrine'.

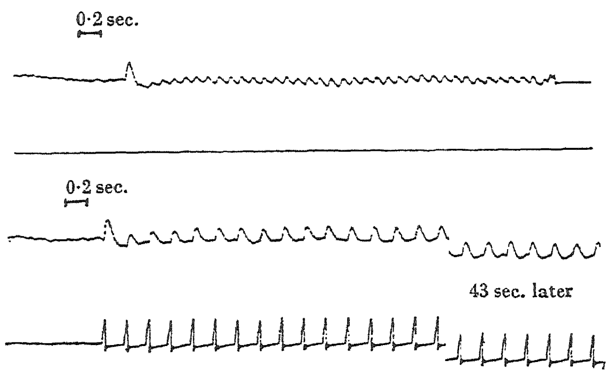

Fig. 19. Initial reduction of the scotopic response followed by return to a steady value. Repeated flashes of violet light (Ilford screen 601, 0·9 e.ft.c.). N.B. The speed of the recording paper is lower in these records than in the other figures.

It is to be noted that with blue light the responses do not become progressively smaller and smaller as stimulation continues. Instead of this the reduction is greatest for the second response, and there is then a progressive return to a steady value, as though the retina discharged its accumulated reserves at the first stimulus, but could then revert to a state of dynamic equilibrium where the supply keeps pace with the demand (Fig. 19). Though

it has not been possible to make continuous records for more than 2 or 3 min. there has been no sign during that period of any alteration in the response after the initial changes in size. This might, perhaps, have been expected in view of the fact that there is no decided reduction in the sensation aroused by the flicker after the first few flashes.

With longer intervals between the flashes, and particularly with yellow, orange-red or white light over a certain intensity range, the reduction of the scotopic component may occur progressively during the first few flashes instead of abruptly (Fig. 20). Records of this are interesting chiefly because they indicate what sort of contribution may be made by the scotopic mechanism to the sensation of colour. With a yellow or orange-red filter there is

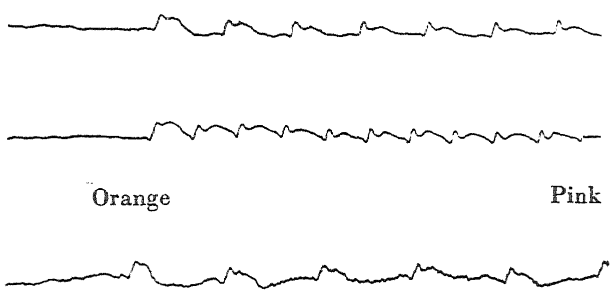

Fig. 20. Progressive reduction of the scotopic component with flashes of orange and orange-red light accompanied by change of hue.

a decided change in hue (from yellow to white and from orange to pink) during the first few flashes, and it is during this period that the scotopic component becomes very much smaller. With the green filters an initial change in hue can sometimes be detected, but it is rarely as clear as with orange.

Discussion

In animals, the dual receptor mechanism of the retina and the complex form of its electrical response are both well known. There have been many comparisons of the response in light- and dark-adaptation and in diurnal and nocturnal animals; light-adaptation has always been found to cause a shortening of the positive wave, and Chaffee, Bovie & Hampson (1923) have suggested an analysis of the frog's electroretinogram which is almost identical with that given here. Yet it cannot be said that a definite separation of the response into a scotopic and a photopic component has been established for any animal but man. In man the dual nature of the response to orange light could scarcely be missed, and the identification of the two components can be made with greater certainty, because there is already so much information about the sensory performance of the scotopic and photopic mechanisms of

the human eye. There is also the advantage that the eye is in a completely normal state during the observations.

It has been found that there are two distinct types of electric response which can be produced in the human eye by a flash of light. One of them behaves in all respects as though it reflected the activity of the scotopic receptor mechanism. It is evoked by light of short but not of long wave-length, it is greatly enhanced by dark-adaptation, and it is absent or very small when the eye is light-adapted. The other type of response seems to reflect the activity of the photopic mechanism, for it is more prominent with bright light, it is evoked by the longer wave-lengths, is mainly derived from the central regions of the retina, and is not much affected by dark-adaptation. At first sight, it is natural to think of these two kinds of response as due to the activity of the rods and cones respectively. For the dark-adapted eye this would be a reasonable conclusion: the photopic type of response is presumably due to a receptor system without visual purple, and in the dark-adapted eye the receptors without visual purple are the cones. In the light-adapted eye, however, we cannot be sure that the response is due entirely to them. It is at least conceivable that the rods can still react to a bright light, but that with the disappearance of the visual purple the electric response set up by a brief illumination of the rods is reduced in size and duration till it comes to resemble that set up by the cones. We may be justified, therefore, in concluding that in the dark-adapted eye the photopic type of response (e.g. that to red light) is due to the cone mechanism and the scotopic type (e.g. to blue) to the rod mechanism, but it is much less certain that the brief responses of the light-adapted eye are due exclusively to the cone mechanism. The brief response after light-adaptation seems to be found in all kinds of vertebrate eye regardless of the proportion of rods to cones; we can say that it must be due to photoreceptors without visual purple, but we cannot be sure that they are all of one histological type. It is for this reason that the terms rod response and cone response have been avoided. It is scarcely necessary to add that the present results do not show whether the potentials are developed in the receptor cells or in some other part of the retinal mechanism.

The photopic response to a brief flash is a diphasic potential change with an initial negativity (which may be very small), succeeded by a brief positive wave. The scotopic response is a larger and longer positive change with no appreciable negativity in front of it. The greater size and different duration might have been expected, but it is more difficult to see why the scotopic response should be monophasic when the photopic appears to be diphasic. One suggestion might be that the different form of the response is caused by the greater concentration of one kind of receptor at the fovea and of the other at the periphery, but this can be tested by confining the stimulus to

different parts of the retina, and it is found that the scotopic and photopic responses retain their usual form wherever they come from. A more likely explanation is that the initial negativity is due to a retinal activity different in kind from that giving the positive phase, for the two phases do not always preserve the same size ratio when the intensity or rate of repetition of the stimulus is altered. This would agree with Granit's analysis of the electro-retinogram into three processes, the first of which produces a negative potential and is not associated with the discharge of impulses in the optic nerve fibres.

Observations with longer periods of illumination must be made before these results can be properly assessed in relation to Granit's analysis, and more accurate recording is needed for precise statements of latency and time relations. But the main purpose of the present experiments has been to investigate the apparent duality of the electric response. If it can really show us how much activity is occurring in the scotopic mechanism and how much in the photopic, it will be worth trying to improve the technique. If it had been found that the two components of the response did not behave in accordance with what we should expect of receptors with and without visual purple, then a further investigation would have much less immediate promise. On the whole, the agreement has been very good. Confirmatory evidence is being sought from other types of eye, and it has been found that the monkey agrees with man in giving records in which the two components can be clearly distinguished. We may conclude that there is a good case for regarding the two components in the human response as due to the scotopic and photopic mechanisms and for investigating them in greater detail.

Summary

1. The electric response of the human eye to a brief flash of light can be separated into two components due to the scotopic and photopic mechanisms of the retina.

2. The photopic component is a brief diphasic potential change. There is an initial negativity of the cornea (which may represent a different form of activity) followed by a brief positive wave. The response is produced by white light or by monochromatic light of all colours except blue. It is present in the fully light-adapted eye and is not much increased by dark-adaptation.

3. The scotopic component is a slower monophasic change reaching 0·3–0·4 mV., with the cornea positive. It is produced by light of all colours except deep red. It is absent in the light-adapted eye and is greatly increased by dark-adaptation. The increase continues for half an hour or more and runs parallel with the increased sensitivity of vision.

4. Deep red light gives the simple photopic response and deep blue light the scotopic. Over the range investigated, changes in the intensity of the

light alter the magnitude of these responses but not the form. This is so for a 100-fold increase with red light and a 4000-fold increase with blue. White light and light of intermediate wave-length give a compound response in which the relative magnitudes of the two components vary with the wave-length and intensity of the light and with the degree of dark-adaptation of the eye.

5. The photopic response is more dependent on the brightness of the central part of the retina and the scotopic on that of the periphery.

6. The scotopic component has not been detected when the flash is thrown on a field which is already lit with a brightness of 2 equivalent foot-candles. It is present when the illumination of the field is reduced to 0·3 e.ft.c.

7. If flashes are repeated at short intervals, the two components are easily distinguished because the scotopic responses may be greatly reduced after the first flash, whereas the photopic responses remain the same size. The reduction is probably due to light-adaptation. It occurs initially and there is usually no further change after the first few responses.

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EXPERIMENTS ON THE HYPOTHALAMIC-PITUITARY CONTROL OF WATER EXCRETION IN DOGS

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The hypothalamus and the pituitary gland have each been shown to play a part in the control of renal water excretion. Frank (1910) first drew attention to the evidence involving the pars nervosa in the production of diabetes insipidus. Camus & Roussy (1913) demonstrated that a lesion localized to the hypothalamus also led to the appearance of diabetes insipidus. That these apparently contradictory findings were in reality compatible has been shown by many physiological, clinical and anatomical findings. Cajal (1894) first showed that the pars nervosa was innervated by fibres arising in the hypothalamus; Greving (1923) traced these fibres to the supra-optic and paraventricular nuclei; and Kary (1924) reported retrograde degeneration in the supra-optic nuclei following injury to the pars nervosa. These findings have frequently been confirmed. The detailed and careful work of Fisher, Ingram & Ranson (1938) finally showed that the pars nervosa and the supra-optic nuclei and tracts must be considered as a single functional unit. Verney (1936) summarized his view by the statement that a normal water diuresis depends upon the integrity of the pars nervosa.

Crowe, Cushing & Homans (1909) were the first to draw attention to the fact that the anterior lobe had a diuretic function.

The experiments to be described in this paper were performed in order to investigate the mechanism which controls the renal excretion of water, and, in particular, the part played by the hypothalamic-pituitary system, including the anterior pituitary. Dogs were, therefore, subjected to various combinations of operations on the pituitary gland and the hypothalamus, in order to analyse, if possible, the part played by each of these structures. The operations were (1) removal of the posterior lobe alone, (2) section of the supra-optic tracts, (3) attempted removal of the anterior lobe alone, (4) simple hypophysectomy, i.e. removal of both lobes together with some of the stalk, (5) simple hypophysectomy and section of the supra-optic tracts at the same operation, thus, it was hoped, destroying the whole pituitary system and most of the related hypothalamus and leaving only a fragment of the pars tuberalis.

A few of the dogs were later treated with dried thyroid (B.P.), suprarenal cortical preparations, and deoxycorticosterone acetate in 20 mg. tablets and 2 c.c. ampoules of 5 mg. in oil. These were used to test the possibility that the changes observed were due to a secondary deficiency in one or other of these glands.

Methods

The bitches chosen were fairly young, up to 4 or 5 years old, healthy, and of all types except whippets and greyhounds. These two breeds were avoided because in the case of one whippet and two greyhounds a transverse venous sinus was found almost immediately below, or just anterior to, the pituitary gland itself, and this made successful operation impossible. The animals were subjected to a preliminary aseptic slitting of the dorsal perineum to facilitate catheterization of the urethra. In two animals the kidneys were denervated by careful stripping of their pedicles and detachment of all peritoneal and capsular connexions with other abdominal structures. The kidneys were finally stitched loosely back in position.

Operations on the pituitary and hypothalamus. These were done through the mouth under nembutal anaesthesia (sodium ethylmethylbutyl barbiturate) as already described for the removal of the posterior part of the gland (Pickford, 1939). Where the anterior part only was removed this was dissected off with a small curved blunt needle and picked out with forceps. In some operations it was found possible to remove the whole anterior lobe in one or two pieces leaving the rounded end of the posterior pituitary standing up like a small collar stud. Great care was taken not to injure the posterior pituitary artery, or to break the stalk. The floor and sides of the sella turcica were always gently scraped lest any piece of gland should be adhering to them. It was inevitable that some of the pars tuberalis should be left intact. When the supra-optic tracts were cut, this was done by a semicircular incision into the base of the brain, between the optic chiasma and the pituitary root, with a cataract knife. This cut was made 2–3 mm. deep and of a transverse width rather greater than the chiasma itself, serial sections of dog hypothalami having shown that this was the greatest distance between the lateral parts of the two supra-optic nuclei. Such an incision, therefore, would certainly divide the cells from their pituitary connexions. For this operation the opening at the base of the skull was slightly forward of the pituitary landmark, so that the optic chiasma was visible and the gland itself neither seen nor touched. Unless otherwise stated, recovery was uneventful.

Collection and examination of urine samples. Over periods of not less than a week at a time, the daily urine was collected from special kennels provided with drainage into jars. The volume per 24 hr., the specific gravity and the chloride concentration of the urine were measured. At intervals the urine was tested for sugar by Benedict's method.

On the occasions when the immediate response to a dose of water or normal saline (i.e. 0·9% (w/v) NaCl) was to be observed, 250 c.c. of water were given by stomach tube in the morning as a hydrating dose, and 2½–3 hr. later 250–300 c.c. water or saline were given. The rate of urine flow was measured by means of a self-retaining catheter draining into a graduated tube.

As a test of their renal capacity for concentrating chloride most of the dogs were given a dose of 3 g. NaCl in 80–100 c.c. water before and after operation. This quantity and volume were chosen as it was found by experience that most dogs could take such a dose without vomiting.

When the creatinine and urea clearances were to be measured, the dog was hydrated as usual; 2–2½ hr. later, 3–3·5 g. creatinine in about 80 c.c. water were given by mouth, and about an hour later the usual 250–300 c.c. water were given. A period of ¾ hr. was allowed for the subsidence of any blood-pressure changes that might follow the administration of the fluid. At least three urine samples were then collected, and at the midpoint of each period a sample of blood was withdrawn from the saphenous vein. This procedure seemed in no way to disturb the dogs. The blood was centrifuged and the creatinine was estimated by Rehberg's (1926) method. Serum urea was estimated by Conway's (1939) method and Cl according to Patterson (1928).

In the urine, creatinine was estimated by Folin's (1914) method, urea by Conway's (1939), and Cl by Smith's (1921) method.

All the creatinine and urea clearances have been reduced to a surface area of 1 sq.m. in order that results from dogs of different sizes may be comparable. This was done with the use of Meeh's formula (Lusk & DuBois, 1924): surface area in sq.dm. $=K^3\sqrt{W^2}$, where $K=11\cdot2$ and W is the weight in kg.

Diet. The diet was a mixed one of biscuits, bread and milk, with meat added when available, and varied within as small limits as possible. A known amount of common salt was added to the food. For the last 4 years it has been impossible to supply more than a small quantity of protein. The maximum daily amount has been about 30 g. protein, but generally for long periods it was nearer 14 g./day. This fact probably accounts for the somewhat low normal creatinine clearances. This explanation is supported by another series of observations in which one dog was fed, in addition to its ordinary diet, 250 g. dried meat daily for 5 weeks, with the result that the simultaneous creatinine/inulin clearances rose steadily from 50 and 49 c.c./min./sq.m. respectively to 94 c.c./min./sq.m.

Examination of post-mortem material. For histological study the animals were rapidly anaesthetized with a 50% mixture of chloroform and ether, both carotids cannulated, and the head washed through with warm saline until the fluid emerging from the external jugular was clear. The hypothalamus and pituitary were then hardened *in situ* by perfusion with 95% alcohol, and, 24 hr. later, dissected out in one piece with the underlying dura. Serial paraffin sections (10–12 μ) were made, and every fifth section kept. Alternate sections were stained with toluidine blue and by Bodian's (1936) silver method. Pieces of the kidney were removed and fixed in 5% formaldehyde before washing out the head. Paraffin sections were prepared and stained with haematoxylin and chromotrope. Except where stated to the contrary, no abnormality in structure was detected.

In most cases both the rostral and caudal supra-optic cells remaining after operation were counted with the aid of an ocular micrometer disk ruled in millimetre squares. In the toluidine blue stained sections, i.e. every tenth section as it came off the block, all cells showing nucleoli were counted. By this method any duplicate counting of cells should be obviated (Magoun & Ranson, 1939). Only those cells were included in the count which had the typical eccentric nucleus and peripheral arrangement of Nissl granules. The same method was applied to the paraventricular nuclei. Here the figures are probably less accurate owing to the rather diffuse boundaries of this nucleus in its postero-lateral area. The figures given in Table 1, then, are not absolute counts, but represent about one-tenth of the whole.

In order to see how much pars anterior and pars tuberalis was left after operation, projection drawings of alternate sections were made in a number of cases. The drawings were then cut out, weighed and compared with those from a whole normal gland similarly drawn and cut out. The figures in Table 1 give the relative weights in paper, taking the weight of the normal anterior pituitary as 100.

Except where otherwise stated, all results mentioned refer to a time when the dogs had reached a stable state, that is, when any temporary effects due to operation had worn off.

For convenience, the word 'diuresis' is used to mean the increased rate of urine flow after water had been given by mouth, and 'polyuria' to mean a greater than normal daily volume of urine.

Results

Before describing any results it must be stated that the attempted removal of the anterior pituitary alone also involved the atrophy of the posterior lobe and a large part of the supra-optic nuclei and tracts, at an unknown interval of time after operation. The reason for this atrophy is not clear, because at the end of each operation the posterior lobe appeared to be in good condition and attached to its stalk, nor was there evidence of damage to the posterior

artery which Wislocki & King (1936) have shown to be the main route for the entry of blood. It may be that, despite the care taken, there was some interference with the blood supply or drainage of the posterior lobe.

After the five operations mentioned, the dogs could be divided into two groups: (1) those which showed a permanently diminished diuretic response to water and no permanent polyuria, (2) those whose diuretic response to water was normal, or greater than normal, and in which polyuria was more or less persistent.

The first type of result followed any of the three operations involving the loss of the anterior pituitary, and was seen in twelve out of thirteen dogs subjected to operation. The second type of result was seen in six out of seven dogs whose posterior lobe had been removed or whose supra-optic tracts had been transected. Only after section of the supra-optic tracts alone was there a prolonged polyuria.

Since a normal pars nervosa system never remained in the absence of the anterior lobe, it is impossible to say whether or not the loss of both lobes is essential for the appearance of a diminished diuretic response. Since, however, almost complete loss of the supra-optico-hypophyseal system and of the posterior lobe is not followed by a decreased diuretic capacity, it is justifiable to assume that the anterior pituitary is chiefly responsible for the effect observed.

The result of loss of both lobes of the pituitary gland

Diminished water diuresis. Simple hypophysectomy was performed on seven dogs, simple hypophysectomy combined with section of the supra-optic tracts in three dogs, and removal of the anterior pituitary alone (with subsequent atrophy of the posterior lobe at a time unknown) in four dogs.

All but one of the dogs in this group showed a reduction in their diuretic response to water by mouth, varying from 15 to 82% of the pre-operative response. The histological material from dog 9 was incomplete, but enough was present to show that there was no pars nervosa left, although a moderate-sized piece of anterior lobe remained. In all other cases the histological material was complete. The findings are summarized in Table 1.

The inability to excrete water rapidly is shown in Fig. 1. Fig. 1*A* shows the response to a single dose of water 34 days after attempted removal of the anterior pituitary alone. Fig. 1*B* shows the result of giving repeated doses of water after simple hypophysectomy. After this operation three doses (300, 300 and 250 c.c.) of water were given at 50 min. intervals, and the rate of urine flow rose to 55 c.c./15 min. Before operation the maximum rate of urine flow was 68 c.c./15 min. after a single dose of water. Fifteen minutes after the third dose the dog showed mild signs of water intoxication. A similar result was obtained in another dog after simple hypophysectomy (No. 47, Fig. 1*C*).

TABLE 1. Summary of histological findings and diuretic capacity

Dog no.	No. of cells in supra-optic nuclei $(\times \frac{1}{10})$	No. of cells in paraventricular nuclei $(\times \frac{1}{10})$	Relative weights of Pars anterior	Pars tuberalis	Pars posterior	% reduction in average maximum rate of urine flow after dose of water
Normal wt., 10·4 kg.	7232	4450	100	2·2	27·7	—
Attempted removal of anterior pituitary alone						
37	277	180	+	+	Trace, atrophied	49
38	428	319	1·7	2·3	Trace, atrophied	41
42	313	152	2·1	0·9	Atrophic	71
43	194	240	A few scattered cells	0	Atrophic	39
Simple hypophysectomy and section of supra-optic tracts						
40	116	c. 100	0	0·45	0	Increased 35%
50	480	150	0	1·38	0	36
52	354	1998	0	+ +	0	71
32*	1439	915	13·6	+ +	Atrophic	64
Simple hypophysectomy						
9	Incomplete	Incomplete	+	+	0	38
14	Lost	Lost	—	—	—	82
18	922	Thinned	Trace	+ +	0	77
19	663	857	Trace	+ +	0	46
34	1326	1326	3·5	+ +	0	46
47	224	376	0	0·9	0	15
Section of supra-optic tracts						
24	196	457	+ +	+ +	Atrophic	Increased 10%
27	244	c. 180	+ +	+ +	Atrophic	Increased 42%
36	381	329	+ +	+ +	Atrophic	0
Removal of posterior pituitary						
10	Lost	Lost	—	—	—	44
16	Incomplete	Incomplete	+ +	+ +	Trace	0
22	327	634	+ +	+ +	0	0
26	861	981	+ +	+ +	0	0

* Cyst of anterior pituitary.
+ Glandular tissue incomplete. + + Glandular tissue complete.

The time of onset of the reduced diuretic response in four dogs after simple hypophysectomy is shown in Table 2.

The first time a poor diuretic response to water was noticed was on the fourth day after simple hypophysectomy in dog 9. One possibility was that, following a temporary polyuria, the dog was in a state of relative dehydration. However, a poor diuretic response was seen even when the hydrating dose was given only $1\frac{1}{2}$–2 hr. before the experimental dose. Since, also, a considerable degree of diuretic incapacity persisted for the rest of the animal's life, it was evident that not temporary dehydration, but some more permanent change resulting from operation was the factor responsible. These findings are, in part, similar to those of White & Heinbecker (1938). These investigators

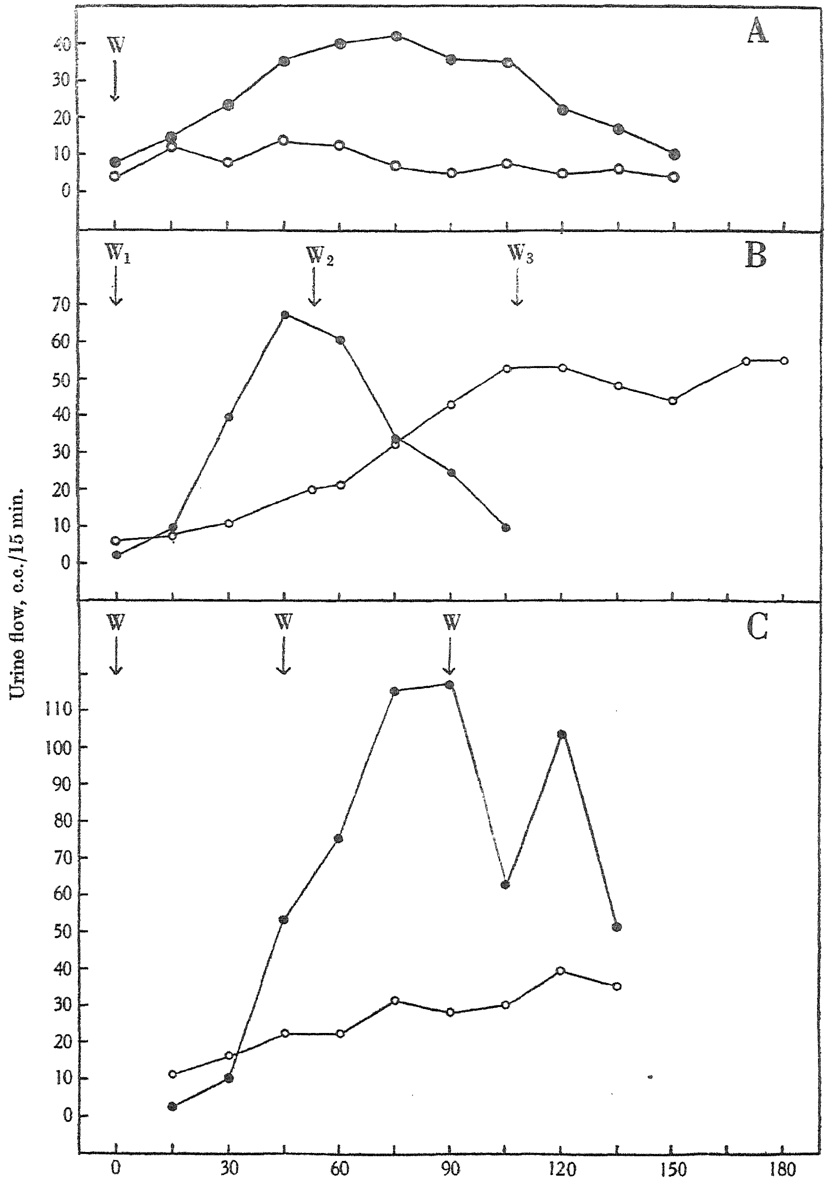

Fig. 1. Diuretic response to water after loss of the anterior pituitary. *A*, dog 42 (anterior lobe removal and posterior lobe atrophy). ●——● before operation. ○——○ on 34th day after operation. At *W* 250 c.c. water given by mouth. *B*, dog 34 (simple hypophysectomy). ●——● before operation. At W_1 300 c.c. water given by mouth. ○——○ on 30th day after operation. At W_1 and W_2 300 c.c. water, and at W_3 250 c.c. water were given. *C*, dog 47 (simple hypophysectomy). ●——● before operation. ○——○ on 14th day after operation. At *W* 250 c.c. water were given.

TABLE 2. Maximum diuretic rate during early days after simple hypophysectomy

Dog 18		Dog 19		Dog 34		Dog 47	
Days after operation	Urine flow c.c./ 15 min.	Days after operation	Urine flow c.c./ 15 min.	Days after operation	Urine flow c.c./ 15 min.	Days after operation	Urine flow c.c./ 15 min.
4	15	3	1·5	1	15	4	103
5	1·5	4	49	2	31	6	50
6	6	58	35	3	29	11	66
7	3	—	—	10	37	14	22
Normal	47		57		74		46

observed a markedly poor diuretic response within 7–10 days after simple hypophysectomy, though in their dogs diuresis was nearly normal 2 weeks after operation.

With regard to the time relations of the water-excretion curves, the maximum, such as it was, was reached after a normal interval. The latter part of the curve was much drawn out, as might be expected with the persisting water load. Denervation of the kidneys did not prevent the appearance of a lowered diuretic capacity (see dog 18, Table 3).

Allied to the diminished diuretic capacity was the ease with which water intoxication could be produced. In one dog (No. 42) as little as 250 c.c. water was found on several occasions to be enough to cause intoxication; the last occasion was 5 weeks after operation. A similar, though less easily produced, intoxication was seen in several other dogs, and showed itself as attacks of barking, defaecation, salivation and convulsions which were first tonic and then clonic. Salivation after the administration of water was frequently observed in all dogs of this group, and varied from an intermittent dribbling to a slow steady stream. The reciprocity of the secretory activities of the kidneys and salivary glands has been observed previously (Klisiecki, Pickford, Rotschild & Verney, 1933; Theobald, 1934).

The effect of these operations on the daily urine volume will be considered in the next section.

Saline diuresis. In five of the fourteen dogs the rate of excretion of normal saline was higher after than before operation; in five it was lower, and in the others it was either unchanged or unknown (Table 3). If the rate of excretion after normal saline is compared with that after water, it will be seen that post-operatively, in eight out of twelve dogs, normal saline resulted in the greater diuresis; the reverse of what is seen in normal animals. This can be seen in Fig. 2 (dog 9, simple hypophysectomy). Eight days after operation water was given, followed 100 min. later by normal saline; 2 days later the order was reversed. In each case the saline produced a large diuresis, but the water had almost no effect on the rate of urine flow.

Creatinine and urea clearances. In nine of the fourteen dogs the creatinine clearances were measured. In seven of the nine dogs the creatinine clearance

TABLE 3

Figures in italics give pre-operative values. Figures in brackets give extreme variations from average. Months or days in brackets give time of observation after operation. The diet for dogs 9, 14, 18, 19 and 28 contained 1·4 g. total Cl per day; the other dogs received 7·5 g. per day.

Dog no.	Post-operative survival months	24 hr. renal water excretion c.c.	Max. urine flow rate after oral adminis-tration of water c.c./15 min.	Max. urine flow rate after oral adminis-tration of normal saline c.c./15 min.	Max. urine flow rate after oral adminis-tration of conc. NaCl c.c./15 min.	Max. urinary Cl after conc. NaCl mg./100 c.c.	Creatinine clearance c.c./min./sq.m.	Urea clearance c.c./min./sq.m.	Body wt. kg.
Attempted removal of anterior lobe alone									
37	4½	*746* 765 (1 month)	*71 (62–81)* 36 (23–55)	*38 (35–39)* 40 (31–52)	— 19	— 700	*80* 43 (15th day) 42 (29th day) 45 (66th day) 38 (136th day)	*50* 31 (15th day) 20 (29th day) 25 (66th day) 22 (136th day)	*15·0* 14·5 (1–4½ months)
38	4½	*414* 853 (1 month)	*44 (38–52)* 26 (18–35)	*33 (32–33)* 23 (22–23)	— —	— —	*67* 38 (11th day) 50 (25th day) 47 (56th day) 24 (134th day)	*30* 39 (11th day) 25 (25th day) 25 (56th day) 13 (134th day)	*7·5* 8·1 (2 months) 9·9 (4 months)
42	2¼	*615* 630 (1 month) 527 (2 months)	*52 (39–66)* 15 (8–23)	*33 (22–44)* 47 (41–53)	*23* 21	*1020* 1030	*85* 45 (47th day) 30 (65th day)	*63* 22 (47th day) 17 (65th day)	*9·75* 9·85 (1–2¼ months)
43	7½	*640* 740 (1 month) 670 (4 months)	*56 (55–57)* 34 (21–39)	*37 (35–39)* 43 (30–48)	*15* 18 (10–37)	*1020* 906	*64* 25 (53rd day) 34 (79th day) 31 (89th day)	*37* 11 (53rd day) 20 (79th day) 20 (89th day)	*9·8* 8·9 (2 weeks–7½ months)

				Simple hypophysectomy and section of supra-optic tracts					
40	2¾	*560* 1087 (13 days) 574 (1½ months)	*37 (36–38)* 50 (41–52)	*11 (5–19)* 41 (36–46)	*8* —	*1385* —	*69* 69 (2nd day) 160 (28th day) 63 (44th day)	*45* 39 (2nd day) 62 (28th day) 38 (44th day)	*6·5* 7·7 (2 weeks–2¾ months)
50	1	*700* 740 (2 weeks)	*47 (46–48)* 30 (27–32)	*59 (32–73)* 22	*15 (11–21)* 35	*900* 580	*50* 34 (20th day)	*23* 20 (20th day)	*7·25* 7·4 (1 week)
52	8	*600* 492 (3 weeks) 371 (4 months)	*61 (37–90)* 18 (5–34)	*36* 41	— —	— —	— —	— —	*7·1* 7·25 (4 months) 6·4 (8 months)
32*	2	*356* 1512 (1 month)	*55 (38–59)* 20 (14–26)	*42 (35–48)* 23 (12–35)	*11–22* 24	*855* 824	*59* 74 (2nd day) 38 (7th day) 48 (52nd day)	— —	*7·8* —
				Simple hypophysectomy					
9	6½	— —	*61 (50–71)* 38 (3–58)	*48 (41–55)* 46 (34–62)	— —	— —	— —	— —	*13·3* 13·5 (1 month) 14·8 (1½ months) 15·2 (2 months) 18·2 (5 months) 19·5 (6½ months)
14	1½	— —	*56 (47–62)* 10 (3–20)	*35* 20 (10–31)	— —	— —	— —	— —	*10·9* 9·6 (1 month)
18	12	— —	*47* 11 (3–22)	— 16	— —	— —	— 38 (284th day)	— —	*6·25* 5·75 (4 months) 6·1 (5 months) 6·5 (6–12 months)
19	3	*315* 595 (3 months)	*57 (48–64)* 31 (22–36)	*40* —	— —	— —	*70* —	— —	*7·7* 7·2 (2 months)
28	3	*180* 673 (1 month) 270 (2 months)	*60* —	— 42	— —	— —	— —	— —	— —
34	1	*280* 965 (1 month)	*74 (61–84)* 40	*51 (48–55)* 42 (33–51)	*27 (25–30)* 15	*720* 508	*52* 56 (16th day)	— —	*14·0* 12·8 (1 month)
47	1½	*696* 1185 (14 days)	*46 (42–53)* 39 (35–44)	*43 (40–46)* 24 (15–33)	*26 (10–40)* 55	*1050* 614	*55* 32 (4th day) 28 (34th day)	*30* 30 (4th day)	*11·7* 12·0 (3 weeks)

* Cyst of anterior pituitary.

was found to be lower than normal after operation, the reduction varying from 32 to 56% (Fig. 3). This agrees with the findings of White, Heinbecker & Rolf (1942), that after simple or total hypophysectomy the inulin clearance was permanently reduced by about 50%. In one of the two dogs (No. 34, simple hypophysectomy) which did not show a reduced creatinine clearance as the result of operation, the maximum rate of urine flow in diuresis was, nevertheless, reduced by 46%. In the other (dog 40, simple hypophysectomy

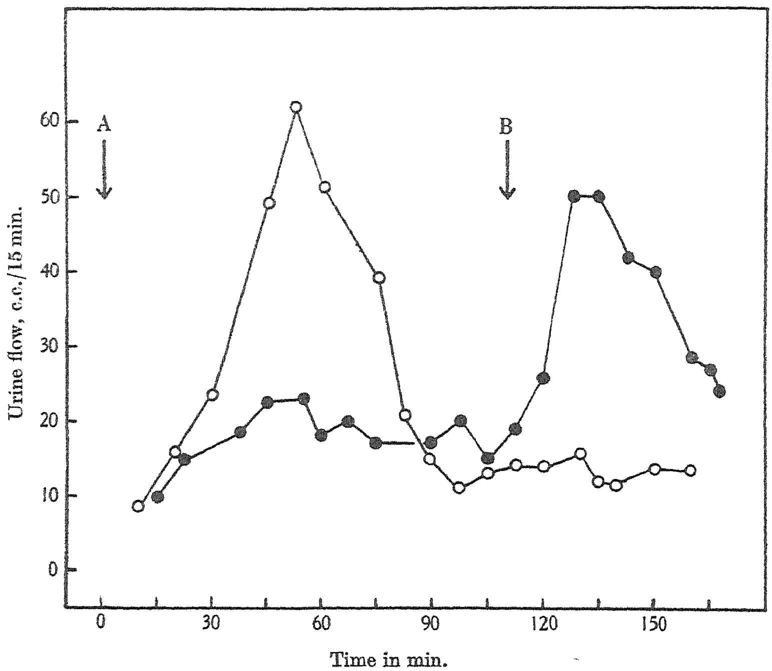

Fig. 2. Diuresis after administration of water and normal saline. Dog 9 (simple hypophysectomy). •——• on 8th day after operation. At *A* 350 c.c. water and at *B* 350 c.c. normal saline. o——o on 10th day after operation. At *A* 350 c.c. normal saline and at *B* 350 c.c. water.

combined with section of the supra-optic tracts), neither the maximum rate of urine flow in diuresis nor the creatinine clearance was lowered. The lowered rate of urine flow in this group does not of itself account for the lower clearance figures, as normal dogs excreting at these lower rates give normal clearance values.

Fig. 4 shows the relation in time between the onset of the diuretic incapacity and the diminished creatinine clearance. It will be seen that the maximum rate of urine flow in diuresis falls before the creatinine clearance is reduced.

The urea clearances were measured in seven dogs. In four of these, operation definitely and permanently reduced the clearance; in three, it was doubtful

if there was any change, and in one of these three (dog 40), the urea clearance was once found to be above the pre-operative level (Table 3).

Blood pressure. Beginning from 2 to 5 days after operation it was found difficult to withdraw a blood sample from the ankle vein. The veins were

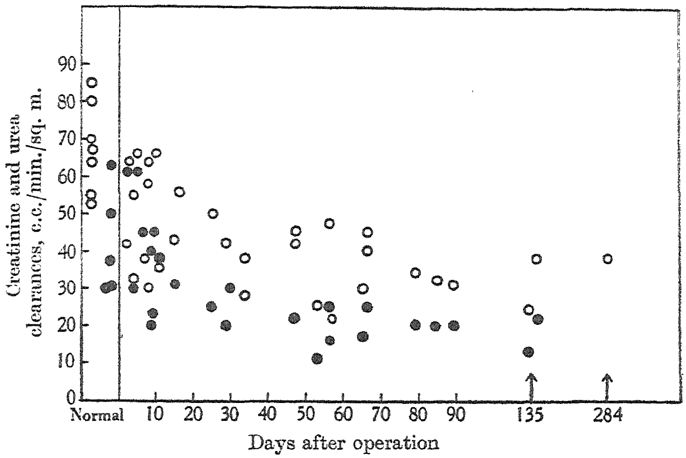

Fig. 3. Creatinine and urea clearances in seven dogs from which the anterior pituitary had been removed, or simple hypophysectomy performed. Open circles, creatinine; dark circles, urea.

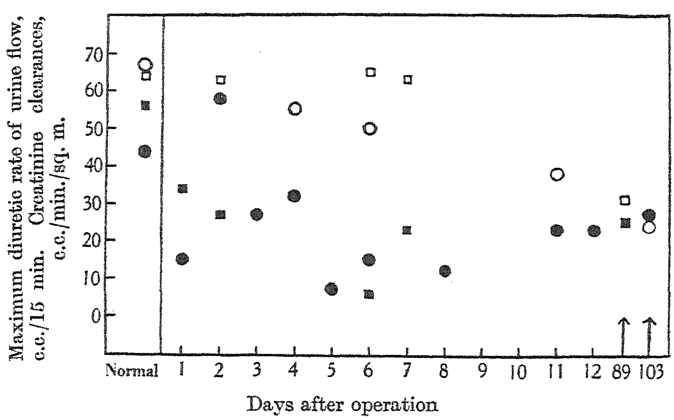

Fig. 4. Maximum diuretic rate of urine flow and creatinine clearances for the first 12 days after the loss of the anterior pituitary with posterior lobe atrophy. Dog 38. Dark circles, urine flow rates; open circles, creatinine clearances. Dog 43. Dark squares, urine flow rates; open squares, creatinine clearances.

collapsed, and distended poorly on the application of pressure. When the needle was inserted, the blood flowed out slowly and seemed both more venous in colour and more viscid than normal. This condition improved 3–4 weeks after operation. The same phenomenon was seen during the normal

interphase (Fisher *et al.* 1938) of those dogs whose supra-optic tracts had been sectioned, and at an analogous post-operative date in those whose posterior lobe had been removed; in both these cases the anterior lobe was left intact. After these last two operations there was, as will be described later, simultaneously with the reduced venous flow, a temporary reduction in the diuretic capacity. These observations suggested that a fall in the arterial pressure might be, in part, responsible for the diminished diuretic response. Five days after operation (attempted removal of anterior pituitary alone), the femoral artery of dog 38 was punctured under local anaesthesia, and the systolic pressure was found to lie between 120 and 130 mm. Hg. This dog was accustomed to being handled and was apparently undisturbed. Four months after operation, the arterial pressure of dog 43 (similar operation) was measured. This dog, too, was used to being handled and showed no signs of disturbance. The systolic pressure was 148–153 mm. Hg. A low general blood pressure, then, does not seem to be the cause of the change in renal activity.

The serum chlorides were estimated from time to time, and it was never found that the pituitary operations had any constant effect on their concentration, nor were the fluctuations observed greater than those occurring in the normal animal. Similarly, changes in the serum urea were small and inconstant. The urine was also frequently tested for the presence of sugar, but none was ever found.

The results in this section may be summarized by saying that, after the three operations involving the loss of the anterior pituitary, there was a diminished diuretic response to water, but the diuretic response to administered saline was variable. In general, saline was, post-operatively, excreted more rapidly than water. The urea and creatinine clearances, after these operations, were diminished, dogs 34 and 40 being exceptions in this respect. Dog 40 requires further comment. This was the first dog in which a simple hypophysectomy was performed and the supra-optic tracts simultaneously cut. This dog seemed to suffer no disturbance in health from the operation, and was as lively as ever the next morning. After operation, the acute diuretic response was above the normal and the rate of excretion of normal saline was increased. This state of affairs persisted for the rest of the animal's life. There was no permanent polyuria. Here, then, was an unimpaired power of excreting water in the entire absence of the anterior pituitary, and with only fragments of the pars tuberalis and of the pars nervosa (the number of supra-optic cells being about 1·6% of the normal). The two other dogs (Nos. 50 and 52) on which similar operations were performed, showed a reduction in the maximum rate of urine flow in diuresis.

Mention must also be made of the exceptional circumstances relating to dog 32. In this animal, whose kidneys had been denervated at a previous operation, the supra-optic tracts were cut cleanly and rapidly, and the

pituitary gland, covered by undisturbed bone, was not seen at operation. From the fourth post-operative day onwards there was a permanent and quite unexpectedly poor diuretic response to water. The creatinine clearance was reduced by 27%. This animal behaved, in fact, as though its anterior pituitary had been at least partially removed. At post-mortem it appeared that the reason for this behaviour might lie in the abnormal anterior pituitary gland. This was swollen to the size of a large pea by a cyst which was covered by a thin layer of anterior pituitary cells, the total number of which was far less than the normal (Table 1). Sections stained with haemalum and eosin, acid fuchsin and aurantia, and with pyrrol blue and eosin showed that these cells were mainly of the acidophil variety; there were a few chromophobes and very few basophils. In consideration of the autopsy findings, this dog is included in the group having loss of the anterior pituitary. Before operation, the response of this dog to water was normal. Since the time of appearance of the lowered diuretic response was similar to that of other dogs which developed diuretic incapacity, it is probable that the anterior pituitary was abnormal, at least from the time of operation. It is possible, therefore, that the cyst resulted directly from the operation, or, if it were already present, that the operation in some way disturbed the functional activity of the remaining anterior pituitary cells.

The post-operative use of thyroid and suprarenal-cortical extracts. Whether or not the thyroid plays a part in the production of diabetes insipidus, it is known to affect the quality of the response to water, and so, in the absence of the anterior pituitary, diminished thyroid activity might be responsible for the low diuretic maxima observed. In normal dogs, the administration of thyroxin leads to an increase in the intensity of the response to water; the rise to the maximum rate of urine flow is more rapid and the maximum itself is higher than the normal (Klisiecki *et al.* 1933; Brull, 1940).

Dried thyroid (B.P., in human therapeutic doses) was given over moderately long periods, as very large doses might merely be adding one abnormality to another. As an example of the effect of treatment, the response of dog 18 (simple hypophysectomy, denervated kidneys) will be given. Eighty-nine days after operation this dog weighed 5·75 kg., as against 6·25 kg. before operation. The dog ate well and seemed well. From the 89th day, after the response to 250 c.c. water had been taken, a single 64 mg. tablet of dried thyroid was given daily for 7 days, and the response to water again measured. For the next 7 days 96 mg. thyroid were given daily and another water diuresis then induced. The results are shown in Fig. 5. It will be seen that the maximum rate of urine flow is greater than before treatment, though it is by no means normal (normal averaged 47 c.c./15 min.), and that the rate of rise to the maximum is more rapid. The resting rate is also higher. These results are similar to those of Klisiecki *et al.* (1933) and Brull (1940) on

normal dogs. The weight of dog 18 had increased to 6·1 kg. Similar results were obtained from three other dogs with innervated kidneys treated with daily doses of thyroid varying from 96 to 192 mg.

In certain animals, such as rats (Smith, 1930), the suprarenal cortex atrophies rapidly after the removal of the anterior pituitary. It was possible, therefore, that dysfunction of the cortex might be responsible for the poor post-operative diuretic response and that treatment with suprarenal cortical extracts, or deoxycorticosterone acetate (DOCA), might improve water diuresis.

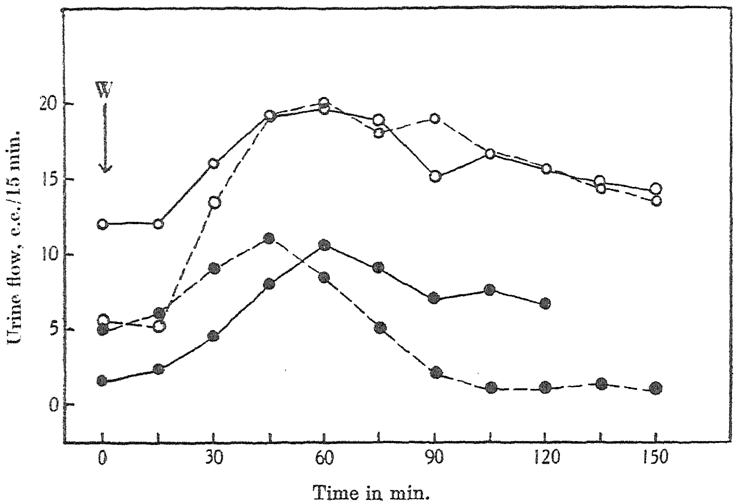

Fig. 5. Effect of thyroid treatment on diuretic response. Dog 18 (simple hypophysectomy). •——• on 89th day after operation and before treatment. o---o on 96th day after operation and after administration of 64 mg. dried thyroid daily for 7 days. o——o on 103rd day after operation and after further 7 days during which 96 mg. thyroid was given each day. •---• on 187th day after operation, 84 days after the end of thyroid treatment.

'Cortin' was therefore given subcutaneously in doses of 2 c.c. per day, for a week at a time, or in repeated doses, both subcutaneously and intravenously, during the morning before diuresis readings were taken. DOCA in oil was used intramuscularly in a similar manner, and tablets of DOCA were also implanted subcutaneously in quantities of 80–160 mg. In only one instance was the diuretic response somewhat increased. The renal clearances showed no return towards normal. In the one instance where improvement was seen (dog 37, attempted removal of anterior pituitary alone), the implantation of 80 mg. DOCA 2 months after operation increased the maximum rate of urine flow in diuresis from a post-operative average of 27 to 40 c.c./15 min. Simultaneous feeding with 192 mg. thyroid daily for 12 days resulted in an average diuretic maximum of 43 c.c./15 min. Once, during the combined

treatment, the diuretic urine flow rate touched 56 c.c./15 min. The pre-operative average had been 71 c.c./15 min. The creatinine and urea clearances were not increased by treatment.

The loss of the pars nervosa. The production of polyuria

After operations limited to the supra-optico-hypophyseal system and posterior lobe there was no permanent diuretic incapacity. A long-lasting polyuria followed section of the supra-optic tracts, but only if anterior pituitary tissue was present. There was no permanent polyuria after removal of the posterior lobe alone.

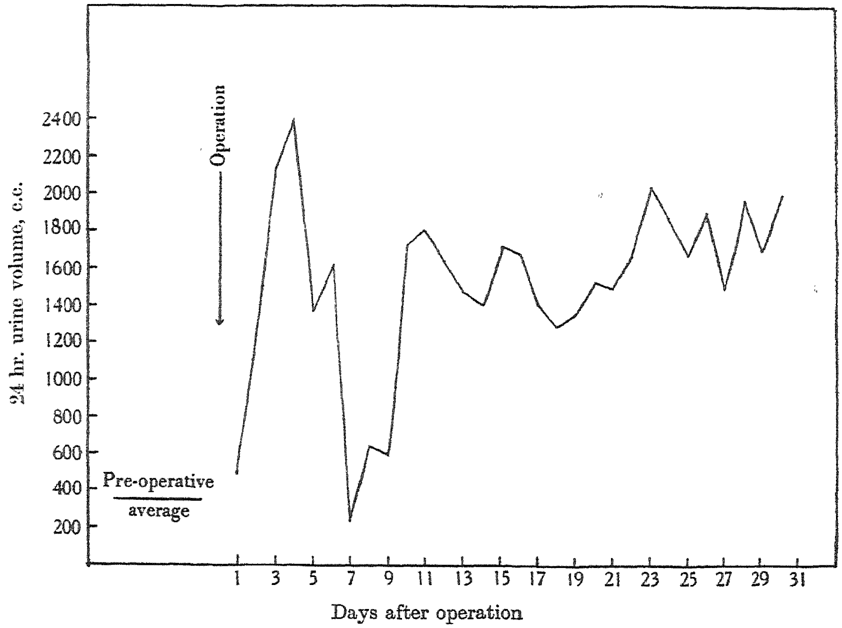

Fig. 6. Preliminary polyuria, interphase and permanent polyuria after section of the supra-optic tracts. Dog 36.

Four dogs (including No. 32, already mentioned, which was found to have a cyst of the anterior pituitary), in which only the supra-optic tracts were cut, showed the expected preliminary polyuria, the normal interphase and then the permanent polyuria (Fisher *et al.* 1938). Fig. 6 is typical of the results obtained. In the only two dogs allowed to survive for some months, the polyuria tended to decrease with the passage of time (Table 4), despite the fact that, in the later stages, the dogs were on a diet containing approximately 7·5 g. Cl per day, as against approximately 1·4 g. pre-operatively and for the first few months after operation.

TABLE 4

Figures and times in brackets as for Table 3. Diet contained 1·4 g. total Cl per day except at periods marked * when it contained 7·5 g. per day.

Dog no.	Post-operative survival months	24 hr. renal water excretion c.c.	Max. urine flow rate after oral administration of water c.c./15 min.	Max. urine flow rate after oral administration of normal saline c.c./15 min.	Max. urine flow rate after oral administration of conc. NaCl c.c./15 min.	Max. urinary Cl after conc. NaCl mg./100 c.c.	Creatinine clearance c.c./min./sq.m.	Body wt. kg.
				Section of supra-optic tracts				
24	11½	*500* 780 (1 month) 850 (4 months) *375 (10 months)	*50 (41–59)* 55 (25–90)	*37 (30–46)* 62 (50–66)	— 36 (26–42)	— 904	*76* 60 (282nd day) 54 (283rd day)	*9·0* 12·0 (3 months) 13·0 (5–11½ months)
27	10	*470* 1870 (1 month) 1100 (2 months) 800 (5 months) *800 (9 months)	*31* 44 (32–59)	— 67 (50–83)	— —	— —	— 62 (30th day) 89 (65th day) 63 (120th day)	*10·2* 12·3 (1 month) 13·5 (8–10 months)
36	1¼	*358* *1700 (1 month)	*42 (35–47)* 40 (39–41)	*45 (35–47)* 51 (41–61)	*22 (20–24)* 57 (51–63)	*1101* 1010	*66* 120 (7th day) 64 (26th day) 43 (38th day)	*7·0* 6·8 (1 month)
				Removal of posterior pituitary.				
10	5½	— —	*41 (31–52)* 23 (16–33)	*35 (30–40)* 42 (32–61)	— —	— —	— —	*10·25* 10·75 (3 months)
16	1½	— —	*52 (44–53)* 51 (45–68)	*25* —	— —	— —	— —	*10·5* 10·75 (1 month) 11·6 (1½ months)
22	19	— 198 (9 months) *721 (15 months)	*42 (36–48)* 44 (29–91)	— 34	— 12	— 1113	— 60 (50th day) 71 (60th day) 50 (80th day) 12 (97th day) 28 (98th day) 63 (452nd day) 67 (483rd day)	*12·3* 14·5 (1½ months) 18·4 (4 months) 15·0 (6 months) 16·5 (11–14 months)
26	14	— 202 (6 months) *1000 (12 months) 221 (13 months)	*61 (54–75)* 60 (51–83)	*12* 34	— 10	— 1175	*61* 51 (14th day) 68 (16th day) 120 (134th day) 67 (390th day)	*12·25* 12·0 (8–11 months) 11·5 (13 months)

Removal of the posterior lobe alone did not result in the appearance of a permanent polyuria. Two dogs (Nos. 22 and 26) did apparently have a 'latent' diabetes insipidus, since, with the Cl intake raised from 1·4 to 7·5 g./day, the volume of urine increased 5 times in dog 26, and 3·6 times in dog 22. On reducing the Cl intake, the urine volume of dog 26 fell to the previous level (Table 4).

The effect of these operations on the number of cells in the supra-optic and paraventricular nuclei may be seen in Table 1. From these figures it seems that a very small part of the whole hypothalamic-posterior pituitary system is enough to prevent excessive loss of water through the kidneys. It will be seen that after a time dog 24, despite the raised Cl intake, became relatively oliguric, and that dogs 22 and 26 passed only small volumes of urine at 9 and 6 months respectively after operation.

A moderate polyuria, lasting up to a month, was seen in some cases after operation involving the loss of the anterior pituitary, but it was not permanent, even where the number of supra-optic and paraventricular cells were as few, or fewer, than after tract section alone. In only one dog (No. 19, simple hypophysectomy) did the polyuria last longer than a month. This dog was being given regularly 500 c.c. water daily by stomach tube, with no other access to water. Before operation, 315 c.c. urine were excreted per day. Three months after operation, and despite the still constant intake, the figure was raised to 595 c.c. This suggests that the dog was polyuric. Another dog (No. 52, simple hypophysectomy combined with section of the supra-optic tracts), on the other hand, far from being polyuric, consistently passed less urine than before operation. It seems that a full and permanent polyuria is impossible in the absence of anterior lobe tissue, though a small degree of polyuria may be seen even in the complete absence of the anterior pituitary (see dogs 40 and 47, Table 3). This is in agreement with the observations of Keller (1938) and of Heinbecker & White (1941).

Only after tract section is there a well-defined interphase, but a temporary polyuria may follow any of the operations mentioned here. When a temporary polyuria does occur, its onset is early after operation, as was observed in dog 37 (attempted removal of anterior pituitary alone). The operation was completed by 5.30 p.m., the dog lying on its side sleeping deeply, with the bladder catheterized. The volume of urine accumulated in the bladder from 2 to 5.30 p.m. was 28 c.c. The volumes of urine passed from 5.30 p.m. onwards were as follows:

5.30–5.45 p.m.	21 c.c.	6.15–6.30 p.m.	35 c.c.
5.45–6.00 p.m.	24 c.c.	6.30–6.45 p.m.	37 c.c.
6.00–6.15 p.m.	31 c.c.	6.45–7.00 p.m.	38 c.c.

Between 7 p.m. and 9 a.m. the next morning, when the dog was cheerful

and lively, 1110 c.c. urine had been passed, no food or drink having been given. Thus, the average rate during the night had been 20 c.c./15 min.

Water diuresis. After section of the supra-optic tracts or removal of the posterior lobe alone, there was no change in the time taken to reach the maximum rate of urine flow in diuresis. After section of the tracts alone, the average maximum rate of urine flow in two dogs (Nos. 24 and 27) was greater than before the operation. This increase persisted until the time of

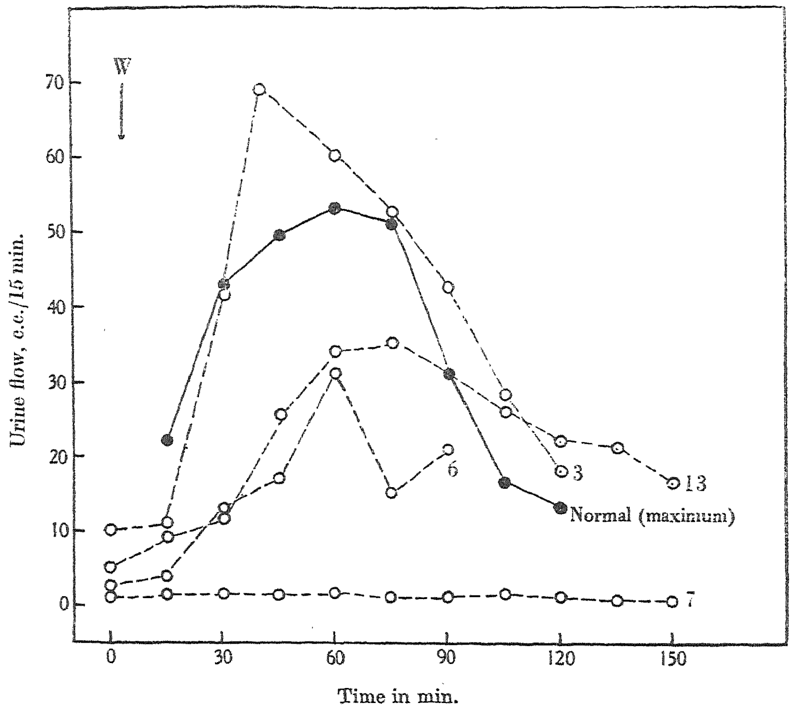

Fig. 7. Effect of removal of the posterior lobe on the maximum rate of urine flow in diuresis (dog 16). At *W*, 300 c.c. water given by mouth. Numbers by the curves give the day after operation on which the observation was made.

death. After removal of the posterior lobe alone, the maximum rate of urine flow was unchanged. In dog 10, however, after an operation at which it was believed that the posterior lobe alone had been removed, the maximum rate of urine flow in diuresis was permanently reduced by 44%. At the operation, in this instance by the temporal route, there was no indication that the anterior lobe had been injured. There was, however, some bleeding which may have been due to injury of the anterior lobe. Unfortunately, the histological material from this dog was lost.

There was always a decreased diuretic capacity during the normal interphase seen after section of the supra-optic tracts, and at a similar post-

operative date in dogs whose posterior lobe had been removed. Fig. 7 shows the response to water of dog 16 for the first few days after removal of the posterior lobe. The antidiuretic phase was, in this case, preceded by a brief phase of increased diuretic capacity (the pre-operative curve in Fig. 7 gives the highest rate observed in fifteen experiments). In dog 22 (similar operation) the order of events was as follows: first to fourth days after operation, antidiuretic phase; fifth to tenth days, increased diuretic maximum (average 69 c.c./15 min.); thereafter the diuretic average was normal, as shown in Table 4.

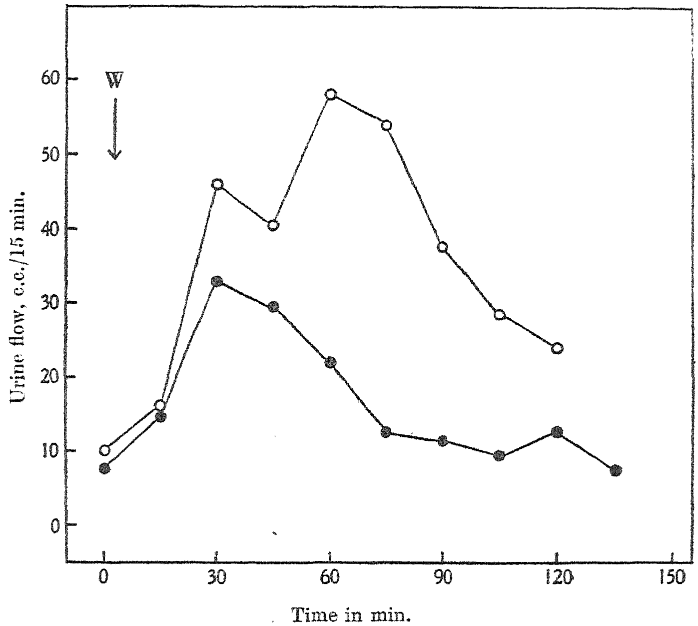

Fig. 8. Diuretic effect of normal saline. Dog 24 (section of supra-optic tracts). •——• before operation. o——o 276 days after operation. At *W* 300 c.c. normal saline given by mouth.

After operations involving the loss of the anterior lobe, the temporary polyuria and the diminished diuretic response appear to be independent of each other. The curves *B* and *C* in Fig. 1, from dogs 34 and 47 (simple hypophysectomy), were taken at a time when these dogs were polyuric (Table 3). Dog 50 (simple hypophysectomy and tract section), for the first 6 days after operation, had a lessened 24 hr. urine volume (517 c.c.), and during those 6 days excreted a dose of water at a higher maximum rate (55 c.c./15 min.) than previously, whilst, after the 24 hr. urine volume had increased, the diuretic response lessened.

After tract section, three of the four dogs (the exception was dog 32 with the anterior lobe cyst) responded to normal saline by a rapid excretion of fluid (Fig. 8). Also, after posterior lobe removal alone, the administration of

normal saline resulted in a greater diuresis than before the removal (Table 4). The total chloride eliminated during the first 2 hr. of the diuresis was, in all instances, within the same limits as before operation.

After section of the supra-optic tracts, fluid was rapidly excreted after administration of hypertonic saline (3%) (dogs 24 and 36). Chloride was concentrated well under these conditions (Table 4).

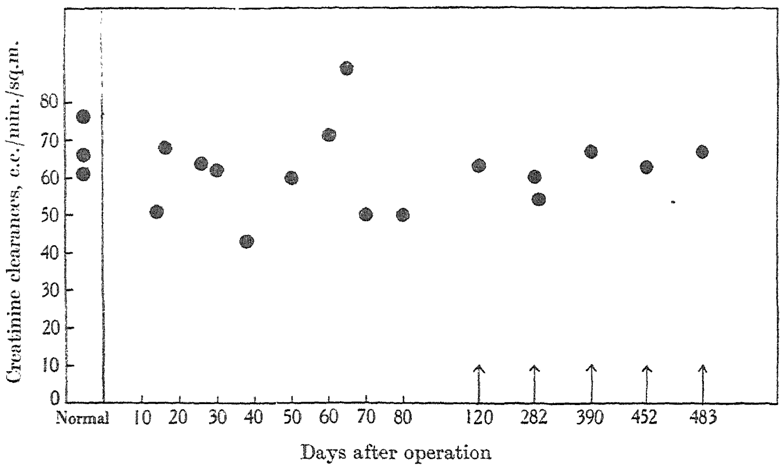

Fig. 9. Creatinine clearances in five dogs after section of the supra-optic tracts or removal of the posterior lobe.

The average creatinine clearance was in the normal range after removal of the posterior lobe, except that there were occasionally wide variations from the average such as were never observed in normal dogs (Table 4, dogs 22 and 26). The observations on creatinine clearance after section of the supra-optic tracts are too few for any definite conclusion to be drawn. Fig. 9 shows that in this group there was, however, no marked reduction of the creatinine clearance such as was seen after loss of the anterior lobe.

Discussion

From the foregoing results it seems that water diuresis, polyuria and the renal clearances may vary independently of each other. Thus, in those dogs in which, during an early period after operation involving the loss of the anterior pituitary, the creatinine clearances were measured, the clearance was found not to vary in direct relation to the diuretic response (Fig. 4); in fact, it fell slowly whilst the poor diuretic response appeared at once, or within a very few days of operation. At this time there might, or might not, be a polyuria.

It was found that the maximum rate of urine flow in acute diuresis was diminished by the absence or reduction of anterior lobe tissue. White & Heinbecker (1938) observed a poor diuretic response for 7–10 days after

simple hypophysectomy and that thereafter there was recovery, though not quite to normal. The results recorded here agree that soon after operation there was a phase of marked anti-diuresis. Later, however, the response to water might, or might not, improve, but in only one case was the maximum rate of urine flow in diuresis found to be more than about 60% of what it had been in the normal condition (Table 1). On the other hand, if the hypothesis of Verney (1936) is correct, the loss of pars nervosa tissue beyond a certain amount should lead to an augmented diuretic maximum. In two instances (dogs 24 and 27), after section of the supra-optic tracts, which left but few surviving supra-optic cells, the post-operative diuretic maximum was above the pre-operative. After operation the diuretic maximum was also consistently increased in dog 40 which had still fewer supra-optic cells and, in addition, complete absence of the anterior lobe. This suggests that in these three dogs there was insufficient pars nervosa tissue for the normal control of an acute water diuresis.

Heinbecker & White (1941) suggested that, early after operation, shock might temporarily paralyse any remaining pars nervosa tissue and be responsible for the resulting preliminary polyuria. On Verney's hypothesis, if the pars nervosa tissue is paralysed, there should be a high maximum rate of urine flow in acute diuresis during the early days after operation. On several occasions this was found to be the case. In dog 24 (tract section), during one period on the morning after operation, the diuretic maximum was 93 c.c./15 min. Two days after simple hypophysectomy a maximum of 103 c.c./15 min. was seen, and on the first day after a piecemeal removal of the anterior lobe alone the maximum averaged 140 c.c./15 min. for 45 min. The same phenomenon is seen in Fig. 7. Thus, high maxima may be seen for a short time after operation irrespective of the presence or absence of the anterior lobe, but, once this brief post-operative phase is over, it does not seem possible to elicit a rapid rate of urine flow in the absence of the anterior lobe.

The only previous workers who have tried to remove the anterior lobe alone are Pencharz, Hopper & Rynearson (1936), who attempted it in rats. Their short communication makes no mention of histological controls. They observed a decrease in water consumption for about a week after operation, followed by polydipsia. Only one dog in the present series (No. 52, simple hypophysectomy and tract section) showed, for 3 days after operation, an immediate decrease in water consumption and this was not followed by polyuria. Dogs, then, do not seem to react in the same way as rats.

A long-lasting polyuria resulted only after operation for the section of the supra-optic tracts. In the absence of the anterior lobe there was, in some cases, a polyuria lasting about a month. This could hardly be due to prolonged shock, and was possibly connected with the posterior lobe atrophy which occurred in every case.

That the creatinine clearance fell after the loss of the anterior lobe is in agreement with the findings of White *et al.* (1942) that the inulin clearance was reduced in similar circumstances. In the present series of experiments the number of observations on the creatinine clearance after supra-optic tract section or removal of the posterior lobe alone are too few to allow any more definite statement to be made than that the average clearance lay in the normal range, in contrast to the fall seen after loss of the anterior lobe.

After loss of the anterior pituitary the administration of thyroid did not result in the reappearance of a normal response to the ingestion of water. This agrees with the observations of White & Heinbecker (1937), who found that the effect of anterior pituitary loss on water excretion was not due to the thyrotrophic factor. Attempted replacement therapy with extracts of anterior pituitary and adrenal cortex met with no success.

Since there is a normal daily loss of water and a decreased diuretic response when the anterior pituitary is absent, it seems that the anterior pituitary is not essential for the former, but is necessary for the production of a normal water diuresis. There is no good evidence that the anterior pituitary releases a specific diuretic hormone in the sense that the pars nervosa produces an antidiuretic hormone. The evidence so far (White *et al.*, 1942) suggests that its absence brings about a change in the vascular condition of the kidney. Whether this effect is direct or through some other organ cannot yet be stated. The anterior pituitary is an aid to the pars nervosa in the production of a normal acute water diuresis since, where a small part of the pars nervosa system is intact, as after incomplete section of the supra-optic tracts, the diuretic response is similar to the normal; where approximately the same number of supra-optic cells are surviving in the absence of the anterior lobe the diuretic maximum is reduced.

The anterior pituitary is also concerned in maintaining normal renal clearances. As the depression of the clearances follows chronologically the depression of the acute diuretic response, the latter cannot be directly dependent on the former. Or, it may be, that diuresis is depressed by a consecutive change in two different factors. Thus, immediately or soon after operation, a dying effort on the part of the pars nervosa may inhibit both polyuria and diuresis. Later, the final disappearance of the anterior lobe factor would, either directly or through some other organ, account for the persistence of an enfeebled diuretic response and, in addition, the appearance of the lowered creatinine and urea clearances. The presence or absence of polyuria would, in the later stages, depend on the amount of pars nervosa left.

The function of the hypothalamic-pars nervosa system is to regulate the excretion of fluid and chloride presented to the kidneys. These two functions may be controlled by different means, since it has been observed (Winter, Sattler & Ingram, 1940) that changes in the one are not necessarily paralleled

by changes in the other. When the pars nervosa substance is greatly reduced in amount, a long-lasting but declining polyuria results. The gradual disappearance of the polyuria suggests that some other process slowly becomes efficient in controlling the volume of water retained in the body. The loss of the major part of the pars nervosa leads, in both water and saline diuresis, to an augmented maximum rate of urine flow which persists till death. The augmented diuretic response to normal saline may be apparent even when the anterior lobe also has been removed.

Dog 40, after removal of all but a fragment of the pars tuberalis and retention of only about 1–2 % of the supra-optico-hypophyseal system, showed but little disturbance of renal function save for an augmented rate of urine flow in both water and saline diuresis. Either this very small part of the whole is sufficient for all the conditions under which this dog was tested, or, alternatively, it must be concluded that there is a reserve control mechanism which is reasonably efficient.

Addendum. The operation for simultaneous simple hypophysectomy and section of the supra-optic tracts appears to be a severe one. Three dogs, not mentioned, died within 3 days from what seemed at the time to be uncomplicated operations. A fourth died on the seventh day with dramatic suddenness after having made an apparently perfect recovery. A fifth (No. 50) died suddenly 33 days after operation. It had been lively and well until one morning when it was found in the kennel in a comatose condition with a pulse frequency of 24/min. and a very slow respiration. 1 c.c. Leptazol (pentamethylenetetrazol, 10 %) and 200 c.c. glucose saline given intravenously improved the condition for an hour or two, but the dog died that afternoon. At post-mortem the only pathological finding was a number of ulcers, shallow and recent, scattered over the pyloric part of the stomach. A sixth dog (No. 47, simple hypophysectomy) also died suddenly, 41 days after operation. It had seemed a little quieter than usual for 2 days, and was found dead the following morning. At post-mortem the only pathological finding was a number of recent ulcers in the pyloric end of the stomach. None of them had eroded through the muscularis mucosae.

Summary

1. The daily urinary excretion of water, and the immediate diuretic response to the administration of water and normal saline (i.e. 0·9 % (w/v) NaCl) have been observed in dogs before and after the following operations: (*a*) removal of the posterior lobe alone; (*b*) section of the supra-optic tracts; (*c*) attempted removal of the anterior lobe alone; (*d*) simple hypophysectomy, i.e. removal of both lobes at the same time and some of the stalk; (*e*) simple hypophysectomy and section of the supra-optic tracts at the same time.

2. After any operation involving the loss of the anterior lobe the diuretic response to water was diminished as compared with the normal. The creatinine and urea clearances also fell. The diuretic response to the administration of normal saline tended to be greater than the response to the administration of water. The blood pressure remained normal. Denervation of the kidneys did not affect the appearance of the changes mentioned. Dried thyroid, anterior pituitary and suprarenal cortical extracts and deoxycorticosterone

acetate were not found to restore the diminished water diuresis, nor the lowered renal clearances, to normal.

3. After section of the supra-optic tracts or removal of the posterior lobe alone water diuresis was normal or augmented. The average creatinine clearances lay in the normal range. The diuretic response to the administration of normal saline was greater than before operation.

4. Polyuria may follow any of these operations, even in the absence of the anterior lobe, but only after section of the supra-optic tracts was it of fairly long duration.

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A STUDY OF THE EFFECTS OF RAPID 'DECOMPRESSION' IN CERTAIN ANIMALS

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Flying at high altitudes produces certain ill-effects on the human body which appear to have a mixed origin. The major changes of environment that might be responsible are the low oxygen pressure, low air temperature, and low barometric pressure characteristic of high altitudes; and to these we may perhaps add, in the case of the airman, continuous vibrations of many frequencies. The ill-effects depend partly on the altitude maintained and the duration, but the rate of change is also found to be important.

Of these factors the reduced oxygen partial pressure is the easiest to understand, and also to compensate, but experience has shown that even the continuous use of pure oxygen at height does not abolish all ill-effects. Between 12,000 ft. (below which ill-effects of any kind are rare) and 45,000 ft. (at which anoxia is apparent even when the gas breathed is pure oxygen), effects are observed which cannot always be attributed to oxygen lack.

Of the remaining factors in the high-altitude environment, the reduced barometric pressure appears able to produce harmful effects even when acting alone, and this fact has naturally directed attention to the experience of deep-sea divers who, though not ordinarily exposed to a lack of oxygen, sometimes suffer ill-effects when returning, or newly returned, to the surface after a dive. The severity of these 'divers bends' has been found by experience to be proportional to (*a*) the working depth of the dive, (*b*) the duration of exposure at the working depth, (*c*) the rate of ascent from the working depth.

It is well recognized that the condition is in the main produced by the formation in the diver's body of bubbles formed from gas which has entered through the lungs under the high pressure at the working depth, and has not had time to escape from the body at the lower pressure of sea-level. As to the factors governing the rate at which ascents may be made in safety, diving experience provides two important data: (1) rapid decompression from 2·1 to 1 atm. is always safe, and (2) for decompressions from pressures higher than 2·1 atm. time must be allowed, depending on the magnitude of the pressure and the duration of previous exposure.

The proper application of this knowledge to the case of the airman raises a number of questions, and the experiments described in this paper were designed to answer some of these. Even the exact application to the diver is a matter on which general agreement has not been reached.

There are two principal schools of thought:

(a) J. S. Haldane (cf. Boycott, Damant & Haldane, 1908) believed that, since instantaneous decompression to 1 atm. from pressures up to 2·1 atm. is safe, the safe limit for a terminal pressure of n atm. is 2·1n atm. He further assumed that the rate of elimination of the excess N_2 is exponential, and he seems to have based his recommendations for divers on the assumed figure of 1% of the excess N_2/min.

(b) Behnke and his associates hold that it is the *difference* between the initial and final pressures in a sudden decompression that is important, and state that this must not exceed 2·3 − 1 = 1·3 atm., if 'bends' are to be avoided. As to the rate of elimination of excess N_2 through the lungs, their measurements indicate it to be a sum of two exponentials, one of which is believed to refer to the 'fatty' tissues of the body and the other to the 'aqueous'. Behnke holds that the decompression process should be so timed that at any instant the pressure of residual N_2 in the body is not more than 1·3 atm. greater than that surrounding the body. We are not able to agree with all the reasoning of Behnke and his associates, but this question is taken up in our discussion.

Our first experiments were concerned with the question of the effects of 'instantaneous' decompression terminating at pressures above and below atmospheric, one object being to decide whether the severity of symptoms was constant or not, when the ratio of initial to final pressure in the system was kept constant.

In other experiments we have measured the rate of elimination of N_2 from dogs exposed abruptly to 1 atm. pressure of pure O_2, since this factor must be important in deciding how soon after decompression the risk of bubble formation passes off. Finally, we have measured the rate of diffusion of N_2 through mutton-fat and olive oil, since this factor is clearly of some importance, and does not seem to have been measured before.

Methods

Decompression experiments

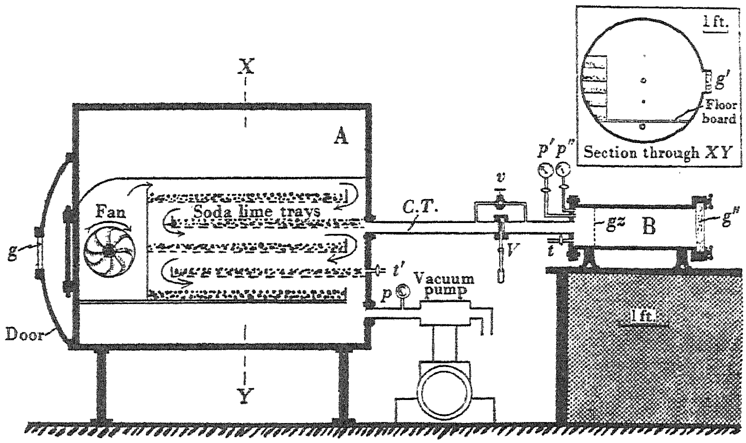
In Text-fig. 1, A represents a large decompression chamber, 5 ft. diam. and 6 ft. long (capacity 120 cu.ft. = 3200 l.), with a 4 ft. diam. door at one end. If necessary, the air within this chamber can be circulated over trays containing soda-lime by a fan (see inset depicting cross-section). Two 8 in. diam. glass windows are fitted (see g' inset) in the walls, and a similar window (g) is located in the door. B is a small chamber 27 by 10 in. internal diameter (capacity 1·25 cu.ft. = 34·0 l.) which can be used as a compression (up to 7 atm.) or a decompression chamber (g'', plate-glass window; p, p' vacuum gauges; p'', pressure gauge). t, t' represent various inlets for the introduction of O_2 or compressed air and for controlling the exit of gases.

The two chambers are connected by a 2 in. diam. pipe ($C.T.$) in which is located a quick-release steam valve (V), short-circuited by a fine-adjustment screw-down valve (v). By means of these valves, the pressure in chamber A can be transferred instantaneously or gradually to chamber B. With V and v closed, the chambers can be used independently. The vacuum pump (Reavell and Co. Ltd., Ipswich, S.A. 6) is capable of lowering the pressure in A to 140 mm. Hg in 5 min. The wire gauze (gz) in chamber B prevents smaller animals from entering pipe $C.T.$, and thus avoids the danger of their being catapulted into the chamber A during explosive decompression.

A third chamber intermediate in size (10 cu.ft. = 300 l.) was used in some experiments, in the place of the small chamber *B*. Each chamber is illuminated by electric lamps in the roof.

The pressure gauges reading above and below atmospheric pressure were calibrated directly against a mercury manometer.

Compression. The animals were placed in the 34 l. chamber, and compressed by means of an electric motor-tyre pump to 6·3, 5·1 or 3·2 atm., pressure levels reached in 6·0, 4·0, and 1·5 min. respectively. Over a 3 hr. compression period the chamber was ventilated by running the compressor every alternate 5 min. period, during which the high-pressure level could be maintained with an open outlet for the escape of accumulated gases (carbon dioxide, water vapour, hydrocarbons). Except during the 'washing through' periods the compressor was only used to compensate for the gradual fall of pressure due to uncontrolled leakage. This amount of 'washing through' was intended to prevent the carbon dioxide partial pressure in the chamber from rising above 10 mm. Hg.



Text-fig. 1. Diagram of apparatus used for the study of the effects of rapid decompression. For details see text.

Decompression. After 3 hr. at high pressure the animals were explosively decompressed by suddenly pulling the quick-release valve. When the final pressure was 1 atm. the animals were taken out of the chamber as quickly as possible, placed in cages, and observed for 1 hr. Those dying were examined as soon after death as the rate of mortality would permit.

Decompression to pressures below atmospheric was carried out by first exhausting the large decompression chamber (*A*) to the required lower pressure and suddenly connecting it to the small chamber (containing the animals), by means of the quick-release valve. At the same time O_2 was run into the small chamber at over 30 l./min., in order quickly to replace all the air in the chamber with pure O_2; after a few minutes this flow was reduced to 5–10 l./min. The animals were observed through the plate-glass door of the small chamber for 1 hr., note being taken of paralysis, general behaviour and of the number dying. At the end of the hour the chamber pressure was raised to atmospheric, and the animals were taken out and examined.

During the hour of observation at the lower pressure, the pressure in the system tended to rise owing to the O_2 inflow; this was compensated for by exhausting the large chamber (still in communication with the small chamber) to the required pressure level.

A copper-constantan thermocouple fitted in the smallest decompression chamber, connected to a mirror galvanometer (the second junction being immersed in water at room temperature) was used to record any transient temperature effects in some experiments involving 'instantaneous' decompression from 1 to 0·16 atm.

Nitrogen elimination measurements

The animals were anaesthetized with intravenous Chloralosane or Pernocton, small additional quantities being injected as the experiment progressed, to maintain anaesthesia. Tracheotomy was performed, and a tracheal cannula inserted. This was connected to a 40 l. spirometer filled with commercial O_2 (N_2 0·5%). The O_2 was kept circulating through an ice-cooled condenser (for moisture) and a soda-lime canister. The temperature, pressure, and volume of the gas in the system were kept under continuous observation, and samples were removed at 1, 3, 7, 10 and 25 min. for analysis (thereafter at hourly intervals). There were no valves in the system: forced circulation was maintained by a centrifugal air pump, and the contents of the spirometer were also stirred by a fan. The electric motors driving the pump and fan were brushless and were wholly within the gas system. No airtight glands were therefore needed.

In starting an experiment the system was flushed out with O_2 and samples analysed for N_2. The dog, after being made to breathe 3–5 times from a bag of pure O_2 (to remove most of the N_2 in the lungs) was attached to the spirometer system.

The amount of N_2 in the closed spirometer system at any moment was calculated from the analysis of a small sample, and records of the temperature, pressure, and volume of the system at the time of sampling. The analysis (Van Slyke & Sendroy, 1932) actually measured N_2 plus any other gas not absorbed by the O_2 absorbing reagents. Such a gas as H_2, for example, if produced in the animal, would be estimated as N_2. (This possibility was investigated separately.) Analyses were always made in duplicate, and duplicates seldom differed by more than 1 part in 250.

The 'zero' of elimination was computed by extrapolation backwards of the first few readings. It was usual to find that, computed in this way, the system (spirometer + lungs) contained at 'zero time' about 300 c.c. of N_2 (measured at N.T.P.). The 'leakage rate' of the apparatus was determined before and after each experiment by attaching an 'artificial lung' ($CaCl_2$ brine, pumped in and out of a bottle attached to the spirometer system). In all but the earliest experiments this rate was less than 1 c.c. N_2/hr.

Measurement of exhaled combustible gases

This test was made by connecting the trachea of an anaesthetized dog to a 200 l. Benedict spirometer, containing O_2 circulating through soda-lime, for 4 hr. A second dog was then similarly connected for a further period of 6 hr. At the end of this time, about 100 l. of gas were left in the apparatus, and this gas was expelled slowly through $CaCl_2$ and soda-lime tubes, to remove any preformed H_2O or CO_2 present, and finally through a tube containing CuO heated to 600° C. Any H_2, methane or other volatile carbon compound would thus be oxidized to H_2O and CO_2 which were trapped in the customary manner and weighed.

Measurement of diffusion constants

The technique used was an adaptation of that employed by Eggleton, Eggleton & Hill (1928) in studying the diffusion of lactate through muscle tissue. A sample of oil (or fat), occupying a measured depth in a cylindrical bottle of about 1 l. capacity, was rendered gas-free by shaking *in vacuo*. It was then maintained in a thermostat for approximately 3 weeks, exposed to pure N_2 at atmospheric pressure. Measured amounts of N_2 were added at intervals to maintain the standard pressure; ultimately, the oil (or melted fat) was shaken with N_2 at the standard pressure and, after equilibration, the solubility of the N_2 was determined in a Van Slyke manometric gas-analysis apparatus.

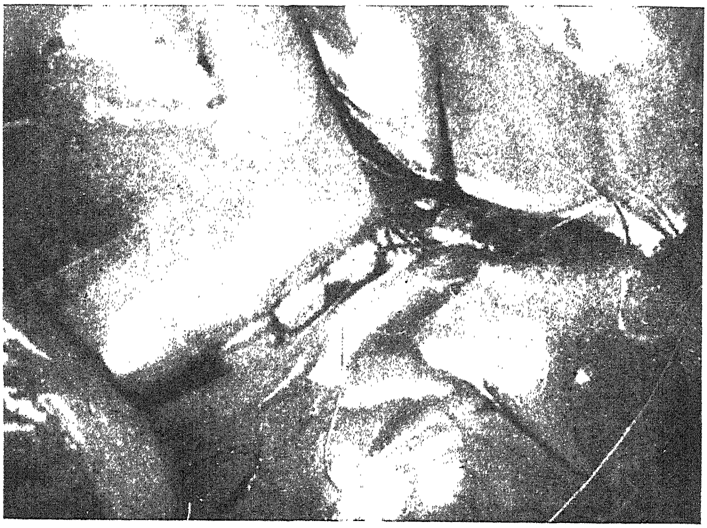


Fig. 1. Gas bubbles in the mesenteric blood vessels of a guinea-pig decompressed instantaneously from atmospheric pressure to 115 mm. Hg. Magnification ×6.

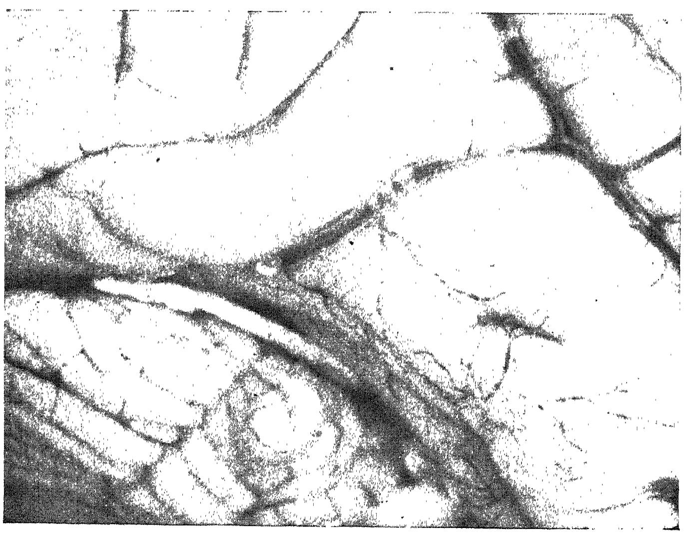


Fig. 2. Gas bubbles in inferior superficial cerebral veins of right temporal lobe of brain in *Macacus rhesus* decompressed instantaneously from atmospheric pressure to 120 mm. of Hg. Magnification ×3.

Results

Effects of 'explosive' decompression over a wide range of absolute pressures, the pressure ratio being kept constant

In our first series of experiments, sudden decompressions were effected from 6·3 to 1, from 5·1 to 0·815, from 3·2 to 0·51, and from 1·0 to 0·16 atm. When the lower pressure reached was less than 1 atm., O_2 was admitted to the chamber immediately after the decompression to avoid the effects of partial anoxia. In this we were probably not completely successful, at least as regards the first few minutes at low pressure.

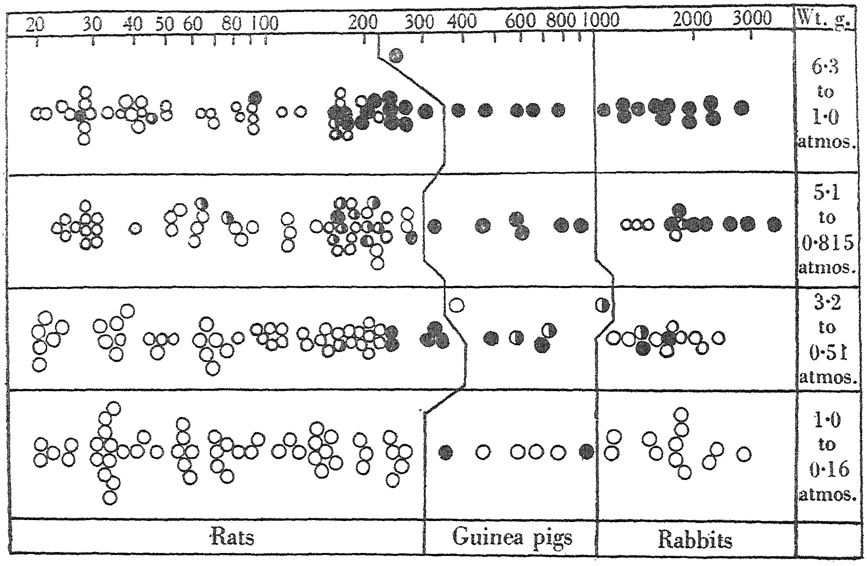
TABLE 1. Effect of explosive decompression over a wide range of absolute pressure, the pressure ratio change being kept constant at 6·3 to 1·0. Observations made within 1 hr. of decompression

	No. of animals	Pressure change in atm. (absolute)	Duration of exposure to higher pressure (min.)	Recovery from paralysis (2)	Persistent paralysis (3)	Death (6)	Score	Percentage score
Mice	71	6·3 to 1·0	147–170	2	1	8	55	13
	60	1·0 to 0·16	∞	0	1	2	15	4
Rats	50	6·3 to 1·0	180	2	1	14	91	30
	50	5·1 to 0·815	180	7	2	2	32	11
	50	3·2 to 0·51	180	1	0	5	32	11
	50	1·0 to 0·16	∞	0	0	0	0	0
Guinea-pigs	6	6·3 to 1·0	180	0	0	6	36	100
	6	5·1 to 0·815	180	0	0	6	36	100
	6	3·2 to 0·51	180	2	1	2	19	53
	6	1·0 to 0·16	∞	0	0	2	12	33
Rabbits	12	6·3 to 1·0	180	0	0	12	72	100
	12	5·1 to 0·815	180	1	0	7	44	61
	12	3·2 to 0·51	180	0	1	2	15	21
	12	1·0 to 0·16	∞	0	0	0	0	0

In Table 1, which summarizes our observations in these experiments, we have adopted a system of scoring, similar to that of Boycott, Damant & Haldane (1908), for assessing the severity of symptoms observed during 1 hr. after decompression. Animals dying were given a score of 6, those paralysed at the end of the hour 3, and those showing paralysis from which they recovered within an hour 2. The percentage score of each group of animals is given in the last column in Table 1. Text-fig. 2 shows the incidence of crippling symptoms and mortality for different weight groups of each species. Table 2 summarizes our observations on the occurrence of gas bubbles in the blood vessels of these animals and Pl. 1, fig. 1 illustrates gas bubbles observed in a guinea-pig decompressed from 1 to 0·15 atm.

The most important result of these observations is that they indicate that the absolute value of the initial pressure is a significant factor in determining the severity of symptoms and mortality in the animal species selected for test.

A certain amount of evidence was obtained that the interval of time between decompression and death in the case of guinea-pigs and rabbits was longer in decompressions from 1·0 to 0·16 atm. than from 6·0 to 1·0 atm.



Text-fig. 2. The ill-effects of rapid decompression. Each individual studied is represented by a circle, the position of which gives the species, body weight, and decompression range (right-hand column). The weight scale (top edge) is logarithmic. A black circle implies death as a result of the decompression; half-black implies crippling or paralysis.

TABLE 2. The incidence of gas bubbles in rapidly decompressed animals

6·3→1 atm.	5·1→0·815 atm.	3·2→0·51 atm.	1→0·16 atm.
	Rabbits		
12 out of 12 died. All examined p.m. All had g.b. + in cardio-vascular system	7 died. All examined p.m. All had g.b. + + 3 survivors not examined p.m.	Both early fatalities and one late death examined p.m. All had g.b. + +	All 8 survivors killed and examined. No g.b.
	Guinea-pigs		
Similar to rabbits	Similar to rabbits	Similar to rabbits	Both animals who died had g.b. at p.m. (not extensive). 4 survivors had no g.b. at p.m.
	Rats		
Of 14 deaths, 10 p.m., all g.b. + + Of 36 survivors, 3 p.m., 1 had g.b.	Of 7 fatalities 2 examined p.m. both had g.b. + + Of 93 survivors, 6 examined p.m., none had g.b.		No p.m. examinations

The animals were decompressed from the higher to the lower pressure shown at the head of each column.
g.b. = gas bubbles; p.m. = post-mortem.

From the results, the following conclusions seem justified, for the given decompression ratio (6 : 1):

(1) Higher absolute pressures result in greater mortality than lower pressures, in all three animal species examined.

(2) Larger rats (presumably older) are more susceptible to the ill-effects of decompression than smaller; this may also be true of guinea-pigs and rabbits, but there is not enough evidence to make this certain.

(3) In all species, fatalities in high-pressure experiments are always associated with gas-bubble formation in the blood. This correlation is less complete in the low-pressure range.

(4) Guinea-pigs appear to be slightly more sensitive to decompression than do rats or rabbits.

It is of interest that of two *Macacus rhesus* decompressed from 1 to 0·16 atm. both died and had gas bubbles in the vascular system. In one case these were confined to cerebral vessels (see Pl. 1, fig. 2). The decompression of another animal of this species from 6 to 1 atm. had produced a similar effect except that it caused the gas bubbles to be more widely distributed through the cardio-vascular system.

The temperature factor. Although a mercury thermometer in the decompression chamber never indicated any lasting change of temperature as a result of decompression, the thermocouple used in some experiments showed a minimum temperature of -30 to $-50°$ C., this minimum being reached in 6–10 sec. The reading returned practically to normal in 60 sec.

Since the true temperature minimum must have occurred within a fraction of a second it is clear that this recording system was not rapid enough, but extrapolation of the temperature curve backwards to zero time indicated a true temperature minimum of the order of $-65°$ C. The adiabatic expansion of dry air to the same extent would be accompanied by a drop in temperature to $-90°$ C., and our smaller observed drop is consistent with the fact that the air was not dry, and that the decompression, though rapid, was not instantaneous.

The anoxia factor. It is instructive to compare the results of these decompressions from 1 to 0·16 atm. with those of a succeeding series in which the final pressure was 0·15 atm., about 8 mm. of Hg lower. In both series an attempt was made to minimize anoxia by admitting O_2 immediately on decompression (initially at 30 l./min., followed by inflow of 10 l./min.). The conditions were therefore identical except for the difference of 8 mm. Hg in the total gas pressure in the chamber. The results are compared in Table 3.

Of the animals dying in these two series, all save one rat proved at post-mortem to have gas bubbles in the cardiovascular system. In the case of guinea-pigs, this manifestation was slighter in the two animals decompressed to 120 mm. than in the remainder.

TABLE 3. The anoxia factor

Decompression	Animals: Rabbits	Guinea-pigs	Rats
To 120 mm. Hg	0/12	2/6	0/50
To 110 mm. Hg	3/12	5/6	10/50

Deaths occurring within 1 hr. of decompression from 760 mm. Hg to the value in mm. Hg given in the first column. O_2 supplied to minimize anoxia.

It seems unlikely that the higher mortality characterizing the experiments at lower final pressure is due directly to the slightly greater decompression ratio; it points rather to an increased importance of the anoxia factor. Analyses made in later experiments of a similar kind showed that the rate of O_2 supply used in these experiments would only maintain the O_2 partial pressure at about two-thirds of the total pressure during the earlier part of the low-pressure period. Such a low O_2 partial pressure is near critical.

Oxygen pretreatment. Since the elimination of excess N_2 from the body takes time, it seems that, in so far as a liability to bubble formation is a factor in the mortality, the risk of death from a sudden decompression exists for several minutes in the dog, and probably for a similar period in the rabbit. We therefore considered the possible effects of replacing the N_2 gas in the animal's body by O_2 before the decompression (a technique first proposed by Zuntz, 1897). An animal which has breathed O_2 for 3–4 hr. will contain very little dissolved N_2, though considerably more dissolved O_2 than a normal animal. Since the solubility of O_2 in water and fat is approximately twice that of N_2 there will be more excess gas to be eliminated immediately upon decompression, but the rapidity with which excess O_2 will be used by metabolic processes should result in the excess of O_2 disappearing in a minute or two. Thus an O_2-pretreated animal, though it may be more prone to bubble formation in the first minute after decompression, should be considerably less prone thereafter.

TABLE 4. Effect of O_2 pretreatment of rabbits on their susceptibility to rapid decompression

	Oxygen pretreated		Not pretreated	
Decompression to	Deaths	Gas bubbles in fatalities	Deaths	Gas bubbles in fatalities
120 mm. Hg	2/14	None	7/24	All had g.b.
100 mm. Hg	8/15	2 had g.b. 6 had no g.b.	14/19	12 had g.b. 2 no g.b.

We accordingly compared a series of rabbits pretreated with O_2 at atmospheric pressure for $3\frac{1}{2}$ hr., with a series of normal rabbits, both series being decompressed as rapidly as possible to 120 mm. Hg. In a second experiment the pressure was reduced to 100 mm. Hg. The results, given in Table 4, indicate that the treatment reduced both mortality and the incidence of gas bubbles in the vascular system.

In similar experiments with guinea-pigs pretreated for 2½ hr. and for 30 min. with pure O_2 the results were similar (Table 5). Although the controls were allowed O_2 from the moment of decompression and thereafter, it is clear that the pretreated animals were better situated during the first minute or so of the low-pressure period in respect of oxygen partial pressure in the surrounding gas.

TABLE 5. The effect of oxygen pretreatment of guinea-pigs on their susceptibility to the ill-effects of rapid decompression

		Pretreated		Controls	
Decompression to	Duration of pretreatment	Deaths	g.b. in fatalities	Deaths	g.b. in fatalities
120 mm. Hg	2½ hr.	0/8	0	4/54	2/4
110 mm. Hg	½ hr.	0/16	0	5/10	1/5

It is therefore arguable that the favourable effect of the O_2 treatment on mortality was due to a lessened incidence of anoxial deaths, but if this be accepted it is then necessary to explain why those pretreated rabbits dying from decompression had a lower incidence of gas bubbles than those untreated rabbits which died. This will be discussed in a later paper.

Nitrogen elimination measurements

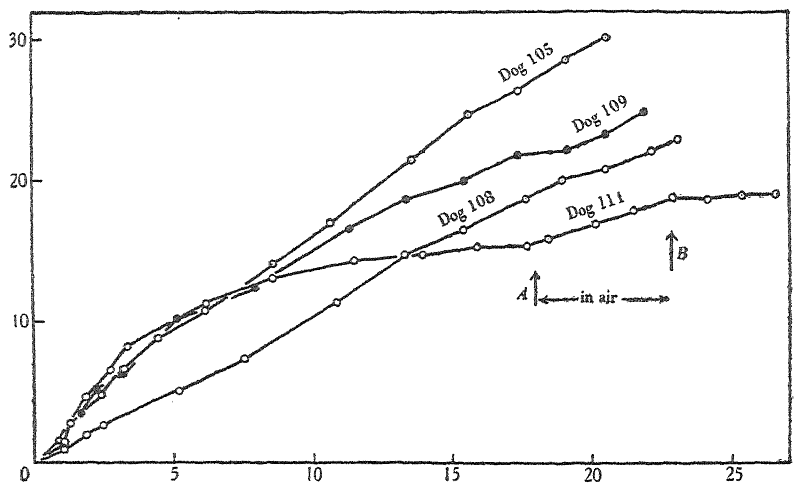
The high correlation of the presence of gas bubbles in the circulatory system with death of the animal corroborates past experience with divers, and focuses attention on the question of the ease with which excess gases in the body can be eliminated from the lungs.

The most extensive study of this process so far made is that of Behnke and his associates, but their measurements on dogs have the drawback that the first 7 min. of the diffusion process could not be followed.

Several workers have studied the elimination of N_2 from human subjects during the first 10 min. of exposure to pure O_2 (Bornstein, 1913; Marshall, Harrop & Grollman, 1928; Campbell & Hill, 1931; Engelhardt, 1939; Darling, Cournand, Mansfield & Richards, 1940; and Darling, Cournand & Richards, 1944). The concentration of N_2 in the expired air drops to half its initial value in a few breaths, and reaches a comparatively steady base-line of 1% in about 40 breaths, or 2–3 min. But the N_2 content of the arterial blood is down to one-twentieth of its initial value in 2 min. (Marshall *et al.* 1928), whence it is clear that elimination from the blood begins well before elimination from the lung cavity is over. In man about one-fifth of the body N_2 is eliminated in 7 min. (see Campbell & Hill, 1931; and Darling *et al.* 1940). With these findings in mind, we made measurements of the rate of elimination of N_2 from dogs exposed suddenly to pure O_2, paying particular attention to the first few minutes and to the final attainment of equilibrium. Although our experiments are few in number, each is as complete as seems possible.

Our results, corrected for the 'zero-time' content of the system, and for the 'leakage rate', are expressed in c.c. of N_2 (N.T.P.) per kg. of body weight, in Text-fig. 3. The abscissa in this graph is the square root of the time (in min.) elapsed. This has the advantage of spreading out the earlier points on each curve, so avoiding congestion near the origin.

Of special interest are the beginning and end of the curves. The elimination in the first 7 min. which has not previously been measured, was 5·6, 3·0 and 6 c.c. N_2/kg. in Exps. 105, 108 and 109 respectively. Dog 105 was very fat,



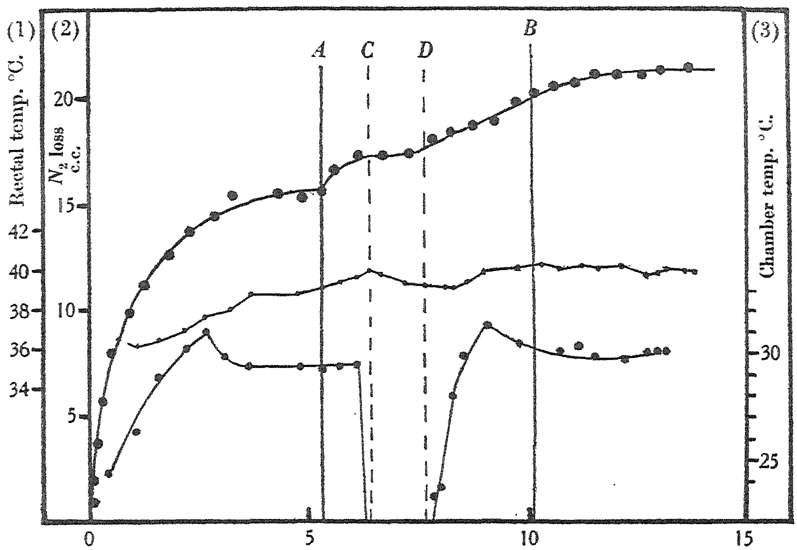
Text-fig. 3. Elimination of N_2 gas from the lungs of anaesthetized dogs exposed abruptly to pure oxygen. Ordinate, N_2 lost in c.c. (N.T.P.) per kg. body weight. Abscissa, square root of time elapsed (in min.). Dog 111 was kept wholly within an O_2 atmosphere; the others remained exposed to air on the outer surface of the body.

and dog 108 enormously fat. The other was normal. The differences are consistent with the idea that N_2 escapes from fatty tissues more slowly than from non-fatty.

With regard to the final stages of N_2 elimination, Text-fig. 3 shows that the rate does not drop to zero even after 9 hr., but falls to a low constant value of 15–30 c.c./hr./kg. This figure is altogether greater than the leakage rate of the apparatus (see Methods), and we have no doubt that it is a true elimination. Three interpretations are possible: (1) N_2 diffuses into the dog from the outer air through the skin and nasopharynx, to be eliminated through the lungs. (2) This N_2 comes from tissues in which conditions are such that it is lost at a steady rate. One such condition is that the N_2 is being formed by metabolic processes from N_2 compounds. (3) This gas is not N_2 but some other gas such as H_2 or CH_4, produced (see Parsons, 1930; Campbell, 1929) at a steady rate and eliminated by the lungs.

Possibility (1) was tested directly in two experiments (111, 117) in which the whole body of the animal lay in a gastight chamber which could be kept flushed with pure O_2 to eliminate the presence of atmospheric N_2. The elimination curve of dog 111 is given in Text-fig. 3.

Exp. 111. After 7 hr. elimination while surrounded by O_2, the dog's N_2 elimination rate was steady at 3 c.c./hr./kg. When air was substituted for O_2 in the chamber (at *A*) the rate rose to a new constant figure of 13 c.c./hr./kg., and dropped again to 3 c.c./hr./kg. when O_2 again replaced air in the chamber (at *B*).



Text-fig. 4. N_2 elimination (upper curve), rectal temperature, and chamber temperature (lowest curve) in Exp. 117. Ordinates, (1) rectal temperature, (2) N_2 elimination in c.c./kg., and (3) chamber temperature. Abscissa, time in hours. For further details see text.

Exp. 117. Dog, 24·0 kg. A preliminary test showed that the N_2 leaking into the apparatus was 1·4 c.c./hr. Extrapolation backwards of the first and third minute samples gave a N_2 content of the lungs and apparatus—and at the time the animal was connected to the closed circuit system containing O_2—of 600 c.c. This value was subtracted from the N_2 content of the closed circuit, subsequently determined. The N_2 elimination curve of this dog is shown in Text-fig. 4. At the end of 5 hr., no further N_2 was being eliminated. The oxygen in the chamber was then replaced by air (*A*) for a period of 5 hr.; during this time 96 c.c. of N_2 (19 c.c./hr.) were eliminated by the lungs. At the end of this time (*B*) the air in the chamber was replaced by O_2. After $2\frac{1}{2}$–3 hr. in O_2, no further elimination of N_2 from the lungs took place.

During the period *C–D* the lid was taken off the chamber to cool the animal. The rectal and chamber temperatures are plotted on the graph. The cooling of the skin surfaces of the animal probably accounted for the depression of the elimination curve in the period *C–D*.

It may be concluded that much if not all of the N_2 which continued to escape from the animal in the earlier experiments was due to the diffusion of atmospheric N_2 into the skin of the animal.

Behnke & Willmon (1941) have published results on man which have led them to the same conclusion, namely, that N_2 diffuses through the skin into the circulation, and is excreted by the lungs. They find that 13–22 c.c. N_2 diffuse per hour through each square metre of body surface with a pressure head of 600 mm. of Hg. Further, they show that the diffusion of helium through the skin increases with the temperature of the skin surface between temperatures of 28 and 36° C. They correlate this finding with the investigations of Hardy & Soderstrom (1938), who found that the blood flow through the skin increases threefold when the skin temperature is raised from 28 to 35° C.

Measurement of elimination of combustible gases

It seemed, nevertheless, desirable to examine directly the possibility (the third possibility already suggested) that some inert gas such as H_2 or CH_4 was being produced at a steady rate, possibly in the intestines of the animal, and eliminated through the lungs. The methods adopted are described on p. 132, and the results are summarized in Table 6.

TABLE 6. Measurement of combustible gases expired by two dogs in 10 hr. For details see text

Volume of O_2 passed through furnace l.	Time taken hr.	H_2O formed by combustion mg.	CO_2 formed by combustion mg.
(*a*) Without any additions (blank tests):			
22·1	45	2·4	1·7
101·1	24	2·9	3·4
(*b*) With petrol ether evaporated into spirometer:			
4·1 (=5·5 mg. ether)	4·5	6·9	11·3
12·1 (=13·4 mg. ether)	16·5	20·5	31·2
(*c*) After rebreathing by dogs, for 10 hr.:			
56·2	19·3	1·5	1·2

There was in 'blank' tests a small production of CO_2 and H_2O, probably from volatile impurities in the lubricating oil of the mixing fan; allowing for this, we could recover approximately 80% of hydrocarbon (petrol) added to the gas in the spirometer, and we are therefore confident that the two dogs studied did not exhale more than 5 mg. of methane or similar gas in the 10 hr. This quantity, if estimated incorrectly as N_2 in the Van Slyke apparatus, would indicate an apparent N_2 elimination of the order of 0·005 c.c. N_2/kg./hr., a negligibly small quantity.

The rate of diffusion of nitrogen through fats and oils

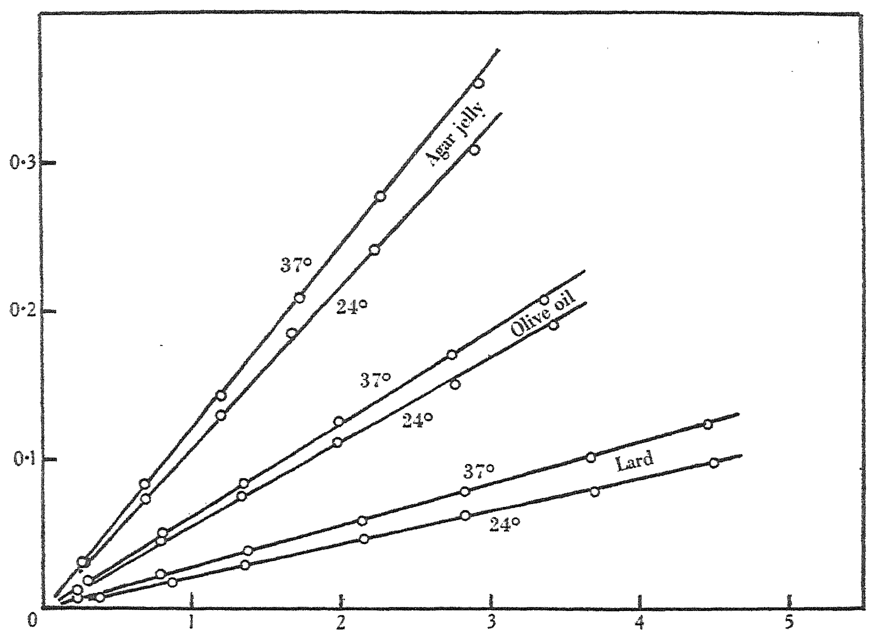
The importance of fatty tissues as a source of dissolved nitrogen in elimination experiments has been emphasized by several writers since Vernon (1907) showed that this gas is 5 times as soluble in oil as in water. Since a proper assessment of the significance of fatty tissues requires also a knowledge of the diffusion constant of N_2 through fats and oils, we have measured these

TABLE 7. Diffusion constants of N_2 in lard, olive oil, and 1% agar jelly

Material	Temp. °C.	Solubility $\times 10^2$	$\frac{2}{D}\sqrt{\frac{K}{\pi}} \times 10^4$	$K \times 10^5$
Olive oil	24	6·8	1·92	0·84
	37	6·7	2·15	1·06
Lard	24	6·5	0·74	0·09
	37	6·7	0·96	0·15
Agar jelly	24	1·5	3·72	2·1
	37	1·2	4·19	2·7

The value of D was 17 cm. for the oil, 14·5 cm. for the lard, and 14·0 cm. for the jelly.
Solubility expressed as the ratio, g. N_2 in solution/g. N_2 in equal volume of gas phase in equilibrium.

constants, being unable to find values in the literature. The measurements were as already described (p. 132), and from the data obtained it is possible to plot the total gas absorbed by the solvent up to a given time, expressed



Text-fig. 5. Diffusion of N_2 into olive oil, lard and agar jelly. Ordinate: amount diffused as fraction of total possible. Abscissa, square root of the time, in days. For other details see text.

as a fraction of the total absorbable, against the square root of the time elapsed. This (see Eggleton *et al.* (1928)) should give a straight line, of slope $\frac{2}{D}\sqrt{\frac{K}{\pi}}$, at least until the diffusion process is more than two-thirds completed. Knowing D, the depth of the oil layer, we can calculate K, the diffusion

constant. Our results are given in Text-fig. 5 and Table 7. There is nothing unexpected in these values, except perhaps in the case of lard, which might have been expected to offer more resistance to the passage of gas than it actually showed. The results suggest that the diffusion constant of N_2 through adipose tissue might be one-tenth of the value in 'aqueous' tissues, but measurements on the tissues themselves are desirable.

Discussion

Rapid decompression experiments

We find that, with a constant decompression ratio of 6·3 to 1, the incidence of crippling and death, and the incidence of gas bubbles in the vascular system, are greatest when the absolute pressure is highest. This is true of rabbits, guinea-pigs and rats. This finding is in direct conflict with the views both of Haldane and of Behnke. Haldane asserted that since rapid decompression from 2·1 to 1 atm. is found safe for divers, rapid decompression from any pressure to a pressure 1/2·1 times as great will be as safe. He did not publish the calculations upon which his recommendations for the safe decompression of divers were based, but we find (see Appendix) that in the case of divers who have been long enough at the working depth to come into equilibrium with the surrounding pressure, his recommendations concerning their rate of 'staged' ascent can be reproduced from the two assumptions that (1) the safety limit for rapid decompressions is that the ratio of initial to final pressure must not exceed 2·15, regardless of the absolute values, and (2) the rate of elimination of excess gas from the body follows an exponential law, one-hundredth of the existing excess being eliminated per minute.

Our decompression results conflict with his first assumption, and in a manner, moreover, that cannot be explained by the partial anoxia which is liable to exist in low-pressure decompressions. Behnke has pointed out that the empirical experience of diving practice is equally well, or better, expressed by the rule that the difference between internal and external pressures should not exceed 1·3 atm. for safety, but this rule clearly breaks down when applied to decompressions starting at 1 atm., since it leads to the conclusion that 'bends' are impossible in decompressions starting from 1·3 atm. or lower. Haldane's two postulates can be applied to this case, however, and lead to the conclusions: (*a*) that abrupt decompression to 20,000 ft. altitude is safe, and (*b*) that subsequently the pressure may be safely lowered at a rate equivalent to 128 ft. of altitude per min. In practice, of course, pure O_2 must be breathed at altitudes greater than 15,000 ft., and this should incidentally accelerate the elimination of nitrogen. Haldane's second rule then leads to the conclusion that a climb at 240 ft./min. is permissible. The actual experience of airmen will thus be a further test of Haldane's views.

We believe that the greater severity of symptoms and greater incidence of gas bubbles in the circulation, resulting from rapid decompressions at higher absolute pressures, may be explicable along lines suggested by J. Piccard (1941), who argued that if a certain minimum number of gas molecules must be aggregated momentarily in a region to initiate a bubble, this minimum local condensation will be more often reached in a solution when the absolute pressure is higher. The evidence of our experiments suggests that the ill-effects of decompression are reduced in subatmospheric tests because the animals are less prone to gas-bubble formation in such tests.

By this last statement we are attaching considerable significance to the results of post-mortem search for gas bubbles which had the highest incidence in tests leading to the greatest mortality and crippling symptoms (that is at high absolute pressures) and the lowest incidence in tests leading to the least mortality, etc. (that is in subatmospheric decompressions). This applies both to the extent to which the cardio-vascular system of any individual animal was affected and to the number of individuals so affected. In decompression from 6·3 atm., animals had gas bubbles; moreover, these were commonly present not only in the systemic vessels, but in the pulmonary and portal systems as well; while in decompression from ground level, with a relatively smaller number of gas bubbles, the bubbles were usually confined to the systemic veins, and only rarely were present in the systemic arteries or the other vessels mentioned. To place too much reliance on this difference in gas-bubble incidence, however, may be unwarranted since the conditions of experimentation also differ. With recompression from one-sixth of an atmosphere to 1, there may be shrinkage, or even disappearance, of some gas bubbles, so that the examination in this respect is not comparable to that made on animals which have not to be recompressed first.

From post-mortem examination alone, then, we cannot be certain that there is a lower incidence of gas-bubble formation in subatmospheric decompression. The results assume more significance from another point of view. If it is considered that air embolism is the primary hazard in high-pressure decompressions, we must conclude that at lower pressures decompression either leads to conditions which allow a more efficient elimination of excess gases, a conclusion supported by the post-mortem examination, or makes air embolism less of a hazard, it being assumed that in the latter case the post-mortem results are misleading as suggested above. Although we regard the second of these explanations as untenable, it is necessary to examine this problem with care, particularly since the acceptance of the first explanation would necessitate a radical rearrangement of our present views concerning the ill-effects of abrupt ambient pressure change.

Nitrogen elimination

With regard to the rate at which excess N_2 is eliminated from the body we think it necessary to criticize the views of Behnke.

Behnke's investigations, with which we include the investigations of his various collaborators, on the dog, led him to formulate three generalizations (1937):

(1) that the quantity of N_2 absorbed by the body when equilibration is reached is proportional to the partial pressure of N_2 in the lungs;

(2) with the same pressure head the rate of N_2 absorption is equal to the rate of elimination; and

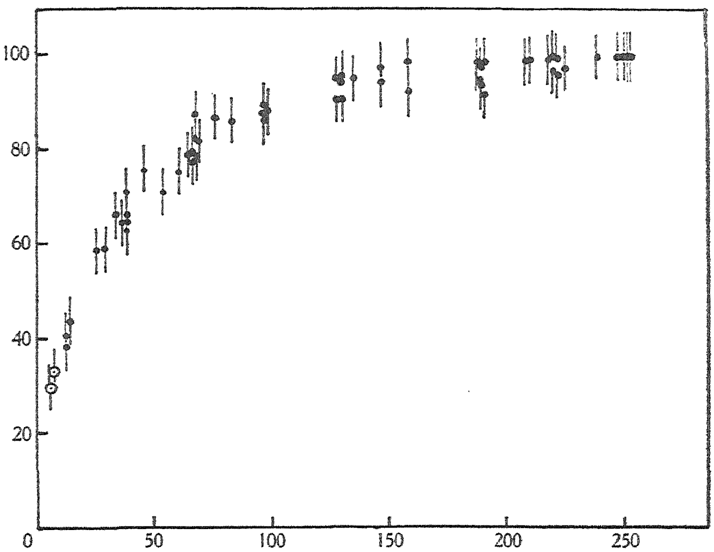
(3) the time required for complete desaturation of the body and the percentage rate of elimination are the same irrespective of the initial quantity of N_2 in the body.

This means that the N_2 elimination curve is of the same shape as the N_2 absorption curve, and both curves are the same shape whether the individual is partially or completely in equilibrium with the initial pressure. That is to say, the percentage rate of N_2 elimination is always the same whatever the initial pressure and regardless of whether the individual had reached equilibrium at that pressure.

Behnke states that 'under conditions of equilibrium at pressures up to 4 atm. absolute the N_2 content of the body is proportional to the partial pressure of N_2 in the lungs'. His basis for this statement is that in a dog kept for 4 hr. at 1, 3 and 4 atm. pressure and transferred in 10 sec. to O_2 at 1 atm. pressure, the total N_2 eliminated between the 8th and 260th minutes was 118, 334 and 500 c.c. respectively, with an admitted error of 5%. These are in the proportions of 1·0 to 2·83 to 4·23, but these figures do not take into account the N_2 lost in the first 8 min. No measurement was made of this amount, but he states that $30 \pm 5\%$ of the body N_2 is eliminated in the first 7 min. Thus the experimental error of the measured N_2 is 5%, and the estimated error of the unmeasured N_2 is 5%, which suggests that the total error will be greater than 5%. We have no reason to doubt the first generalization, but consider that it is not very rigidly demonstrated. We will show later that this estimate of the error involved is not exaggerated.

With regard to Behnke's second and third generalizations, their acceptance must also stand or fall upon the degree of accuracy with which he is able to measure the curve of N_2 elimination. If his generalizations are true, then all the N_2 elimination curves which he publishes in support of his thesis—when plotted as percentages of the total N_2 eliminated and with the time scales adjusted to make all curves coincide at one point—should coincide at all points within the experimental error of the apparatus. To test this we have replotted all the curves of nitrogen elimination of dogs A, B, D, E and G

(Behnke, Thomson, Messer & Motley, 1935) in the following manner. The total amount of N_2 eliminated in each experiment was taken as 100%, and the unmeasured amount of N_2 eliminated in the first 7 or 8 min. was taken as 30 or 32% respectively. The intermediate points were then plotted on this basis (see Text-fig. 6). Taking Behnke's own statement that in the region of 30% elimination the error is 5% and that a similar error affects all his estimations, we have represented this by marking each experimental point as a line 5 units above and below the observed point. In order to avoid confusion we give in the figure only the dogs mentioned; it should be said, however, that the following assessment applies equally to the other dogs.



Text-fig. 6. N_2 elimination curve of dogs based on measurements of Behnke and collaborators. Ordinates: N_2 eliminated as a percentage of total lost. Abscissa: time in min.

An examination of our Text-fig. 6 shows that the measurements on which Behnke's generalizations are based are only true to within not less than 7%, and the question arises as to whether such approximate generalizations are of any practical use. Within the envelope of the experimental points referring to dog D, several curves of quite different mathematical properties, for example an inverse tangent, a rectangular hyperbola and a simple exponential, etc., may be fitted with equal propriety. This being so, it is valueless to express mathematically, as Behnke has done, the results of N_2 elimination experiments when such a large error of this order of approximation does not justify generalizations, such as Behnke's second and third, so difficult to reconcile with our knowledge of physiological mechanisms.

It should be stressed, however, that, except in one respect, it is doubtful whether the degree of accuracy attained by Behnke can be improved upon. If not, then N_2 elimination experiments will throw little further light upon the nature of N_2 exchange within the body and to the outside air during decompression. With regard to the exception, Behnke's experiments on dogs did not measure the first 30% of the N_2 exchange, and his conclusions as to the reversibility and as to the non-necessity for complete equilibration refer only to the last 70% of the curve. In his experiments on man he was unable to measure the N_2 eliminated in the first 5 min., and his experiments were restricted to simple N_2 elimination in O_2 at 1 atm. and therefore give no information as to the identity of elimination and absorption curves or the effect of incomplete equilibration.

When an analysis is made of Behnke's interpretations of experiments on the dog and the attempts made to apply them to man, the lines of reasoning he adopts seem to be inconsistent. In the first paper of Behnke, Thomson, Messer & Motley (1935) it is concluded from experiments on dogs that the N_2 desaturation time is constant and irrespective of the degree of saturation, and they state 'this indicates that the N_2 in the quickly saturating tissues is constantly moving into the more slowly saturating tissues, thus tending to equalize the N_2 tension throughout the body at all times'. Thus they infer that the rate of desaturation of the slowly equilibrating tissues is indistinguishable in the elimination curve from the rate of desaturation of the quickly equilibrating tissues. Paradoxical as this appears to be, they leave no doubt in the mind of the reader that this is their view, for in the last paragraph of the paper we find the statement 'that complete desaturation from a state of partial saturation follows the same curve, and consequently requires the same length of time as desaturation from a state of complete saturation'. It appears to us that the nett result of their views might be expressed by saying that there is in practice no necessity to distinguish between slowly and rapidly saturating tissues, for the N_2 equilibrium in all tissues is so rapidly attained that the body for N_2 elimination experiments can be considered as a homogeneous system.

In a second paper Behnke, Thomson & Shaw (1935) state explicitly that they present 'the application of certain principles from studies of the dissolved N_2 in dogs to measurement of N_2 in man'. They give a total N_2 elimination curve (Fig. 1 A of their paper) typical of the results from twenty-five experiments on three healthy men, and then explain that the total N_2 elimination curve can be conveniently represented by two exponential equations of different slopes representing 'fat' and 'water' elimination curves—the sum of these two curves being the total N_2 elimination curve. These two curves are constructed from calculations based upon the fact that the solubility of N_2 in fat and lipids is 5–6 times greater than in water (Vernon, 1907), and the

assumption that the N_2 removal from fat and lipids will take 10–12 times longer than N_2 removal from body fluids. No account, however, is taken of the fact that the blood supply to the 'water' tissues differs markedly from that to fat tissues. In this second paper, therefore, there is, on the basis of the previous work of others, a tacit acceptance that in man the rates of N_2 elimination from quickly and slowly saturating tissues separately determine the shape of the elimination curve. It is clear that the definite opinion expressed regarding the constancy of the N_2 desaturation time irrespective of the degree of saturation in respect of dogs cannot be reconciled with their later view that the N_2 elimination curve in man is the sum of two curves of different slopes representing N_2 elimination from the quickly and slowly saturating tissues: therefore, on his own showing, Behnke's last two generalizations derived from the dog experiments are not applicable to man.

Our own elimination measurements do not fit well a simple exponential rule of elimination. They could, of course, be fitted to the sum of two or three exponentials by appropriate choice of values for the four or six constants involved, but this would prove nothing. Actually, the average elimination curve of our five dogs, when corrected for the 'skin leakage', is practically linear with respect to $\sqrt{}$time for the first half of the process. Such a relation is not uncommon where diffusion is concerned, but does not necessarily have any significance in this case.

Summary

1. Rats, guinea-pigs, and rabbits were 'decompressed' rapidly to a pressure approximately one-sixth of that with which they were in equilibrium. At the same time, when necessary, the atmosphere was enriched with O_2 to avoid anoxia. The animals were under observation for an hour at the lowered pressure. It was found that:

(*a*) Experiments at higher absolute pressures resulted in higher mortality than experiments at lower absolute pressures, in all three animal species.

(*b*) Larger (and presumably older) rats are more susceptible to the ill-effects of decompression than smaller rats.

(*c*) In all species fatalities in high-pressure experiments are always associated with gas-bubble formation in the blood. This correlation is less complete in the low-pressure range.

(*d*) Guinea-pigs were more susceptible to decompression sickness than rats or rabbits.

2. Three monkeys (*Macacus rhesus*) treated similarly all died, and bubbles were present at post-mortem in the blood of each. One, decompressed from 6·3 to 1 atm., had more widely distributed bubbles than the other two, which were decompressed from 1 to 0·16 atm.

3. Rats, rabbits and guinea-pigs decompressed from 1 to 0·15 atm. suffered considerably greater mortality than animals decompressed from 1 to 0·16 atm.,

although the atmosphere was enriched with O_2 at the lower pressure in both cases. Of the rats that died all save one proved at post-mortem to have bubbles in their blood.

4. It was found that rabbits and guinea-pigs which had breathed pure O_2 for 3–4 hr. previously to decompression (from 1 to 0·16 atm.) were less affected than animals not so pretreated. Gas bubbles were less in evidence at post-mortem.

5. Measurements of the temperature in the decompression chamber, made with a thermocouple, indicated that the rapid reduction of pressure from 1 to 0·16 atm. was accompanied by a drop in temperature of at least 40–60° C. The temperature returned to normal in about 1 min.

6. Measurements were made of the rate of elimination of N_2 gas from anaesthetized dogs exposed abruptly to O_2 at 1 atm. pressure. When only the lungs were thus exposed there was evidence that N_2 entered from the air through the skin of the animal at a rate of about 15–30 c.c./kg./hr. Apart from this source of error the elimination occurred through the lungs at the rate of 8–12% of the total originally in the body, per 'root minute', until 50–70% of the elimination was over.

7. Measurements of the diffusion constant of N_2 through agar jelly, olive oil, and lard, have been made, for temperatures of 24 and 37° C.

This work was carried out at the instigation of Prof. I. de Burgh Daly, without whose help and advice in the design of apparatus and of experiment much of the work could not have been done. To him, and to Dr W. O. Kermack, who gave generous help in the mathematical aspects of the problem, we are glad to express our deep indebtedness.

We are indebted to Air Marshal Sir Harold Whittingham, Director-General of Medical Services, R.A.F., for permission to publish these results which were initially sent in as Progress Reports to the Flying Personnel Research Committee, *Reports* Nos. 200 (1940), 316 (1941), 349 (1941), 495 (1942) and 541 (1943). The cost of investigation was borne principally by the Air Ministry and by the Moray Endowment Research Fund.

APPENDIX

The 'staged' decompression introduced into diving practice by Haldane consists in raising the diver rapidly to a point where the hydrostatic pressure (and therefore the air pressure in his helmet) is $1/R$ times the pressure at the working level. Here the diver has to wait until the elimination of excess gas from his tissues has advanced to the point at which it is safe to raise him a further 10 ft.: that is, until his internal gas pressure has fallen to R times the hydrostatic pressure 10 ft. higher up. It is then safe to raise him this 10 ft., where he must wait again. In this way he proceeds by 10 ft. 'stages' to the surface.

If Haldane's suggested times of decompression at each level are based on the assumption of a logarithmic rate of equilibration, then for any such stage we have

$$\frac{\text{final internal pressure} - \text{external pressure}}{\text{initial internal pressure} - \text{external pressure}} = e^{-Bt}.$$

Now Haldane's decompression levels are all multiples of 10 ft. depth, so that the external pressure is $1+n/3{\cdot}3$ units (n being the stage number counting from the top downwards). Further, for all

except the first stage of an ascent, the initial internal pressure is R times this figure (by Haldane's rule), and the final internal pressure is R times the external pressure 10 ft. higher. Hence

$$\frac{R\left(1+\frac{n-1}{3\cdot 3}\right)-\left(1+\frac{n}{3\cdot 3}\right)}{R\left(1+\frac{n}{3\cdot 3}\right)-\left(1+\frac{n}{3\cdot 3}\right)}=e^{-Bt},$$

or

$$\frac{R}{(R-1)\,(n+3\cdot 3)}=1-e^{-Bt}.$$

For the first stage this will not, in general, be true. The diver's internal pressure at the beginning of the first halt will be that of the sea bed he started from, or $1+\frac{D}{33}$, where D was the depth of the sea bed.

For the first decompression stage therefore

$$\frac{R\left(1+\frac{n-1}{3\cdot 3}\right)-\left(1+\frac{n}{3\cdot 3}\right)}{\left(1+\frac{D}{33}\right)-\left(1+\frac{n}{3\cdot 3}\right)}=e^{-Bt},$$

or

$$\frac{(R-1)\,(33+10n)-10R}{D-10n}=e^{-Bt}.$$

The only entries in Haldane's tables that are of interest in the present connexion are those in which the diver has been long enough at the working depth to become equilibrated with it; that is, for a period so long that Haldane does not need to specify the exact duration, and the problem is to find whether values of R and B exist which will lead to the same values of all these decompression times. We find that by assigning the values $R=2\cdot 15$ and $B=1/100$ a tolerably good reproduction can be made of them. The agreement holds over the whole range of the table except in respect of the final decompression, where our figures require a longer equilibration period. If our reconstruction is valid it would seem that Haldane deliberately abbreviated the final equilibration period by 10–20 min. because the danger at this stage is least. Any bubble formed is not acted upon by any further decompressions.

It may therefore be concluded that according to Haldane's view an airman might safely ascend at such a speed that at all instants the atmospheric pressure with which his body would be in equilibrium (p) is 2·15 times the actual atmospheric pressure (π) around him. He could, of course, rise instantly to the altitude of 18,000 ft. at which point the value of p/π reaches Haldane's limiting ratio R ($=2\cdot 15$). From then on, his limiting rate of ascent would be a constant velocity of about 130 ft./min.

The argument is as follows. The atmospheric pressure (π) falls off with altitude approximately according to a simple exponential law $d\pi/dh=-A\pi$, in which $A=0\cdot 042$ if the height (h) is in thousands of feet. N_2 is assumed to diffuse out of a man's body at a rate proportional to its excess pressure ($p-\pi$) over the surroundings, i.e. $-dp/dt=B\,(p-\pi)$. Finally, the condition set in this limiting rate of ascent is that p/π starts with the value R and keeps it:

$$\frac{p}{\pi}=\frac{dp}{d\pi}=R$$

Now speed of climb

$$\begin{aligned}&=\frac{dh}{dt}\\&=\frac{dh}{d\pi}\frac{d\pi}{dp}\frac{dp}{dt}\\&=-\frac{1}{A\pi}\frac{1}{R}-B\,(p-\pi)\\&=B\frac{(R-1)}{AR}.\end{aligned}$$

Taking $B=0{\cdot}01$, $A=0{\cdot}042$, $P=2{\cdot}15$;

$$\text{Limiting speed}=\frac{1000\times 1{\cdot}15}{100\times 0{\cdot}042\times 2{\cdot}15}=128\text{ ft./min.}$$

If the man breathes pure O_2 throughout, his rate of elimination of N_2 is greater, being no longer retarded by the N_2 partial pressure in the surrounding atmosphere. The situation is that $dp/dt=-Bp$, and the limiting velocity no longer depends on R. It is now simply B/A, or 240 ft./min.

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THE EFFECT OF CUTANEOUS BURNS ON HISTAMINE IN MICE

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The effect of cutaneous burns on the histamine content of the tissues is interesting because it is possible that histamine plays some part in the causation of 'shock' after burns. This shock may be entirely caused by the local loss of plasma at the site of the burn (Rossiter, 1943); or, alternatively, the absorption of toxic substances into the general circulation may play some part in its causation (Moon, 1942). The formation, or liberation, of histamine might act either by increasing the permeability of the capillaries in the skin itself, or by modifying the circulation as a whole after absorption.

The possible significance of histamine in injuries was first suggested by the work of Dale & Richards (1918), and Dale & Laidlaw (1919) on the dramatic effects produced by the intravenous injection of small doses of histamine. It received support from Lewis & Grant (1924, 1926), Lewis & Harmer (1927), and Lewis (1927), who obtained evidence of the liberation of a histamine-like substance on irritation of the skin. When histamine was shown to be a normal constituent of the body (Best, Dale, Dudley & Thorpe, 1927; Dale & Dudley, 1929; Thorpe, 1928, 1930), it seemed highly probable that this very active substance played an important part in the response of the body to injury, but the evidence on this point is still inconclusive.

Histamine is not the only substance which may be liberated by injury; about twenty other different substances have been suggested as possible causes of shock, or toxaemia, following burns (Harkins, 1942). Histamine is, however, of special interest because it causes shock in much smaller doses than any other substance which has hitherto been isolated from tissues. It has the advantage, as an object for experiments, that, with suitable precautions, very low concentrations in tissues can be accurately measured by biological assay. This is only possible if the extracts are treated in such a way as to remove, or destroy, all other substances with an action on the pharmacological test used for the assay. The experiments may therefore miss some other toxic substance which plays a more important part than histamine in the causation of shock, but this limitation increases their precision.

It is generally agreed that extracts of skin normally contain histamine. The first experiments on the effects of burns on this histamine were carried out by Harris (1927), who used alcoholic extracts and compared them directly with histamine for their effects on the blood pressure of cats. He found that when the skin of an anaesthetized cat was burned with a hot flat-iron there was oedema, but no change occurred in the histamine equivalent of a given area of skin during the first hour. After this time, the histamine and the oedema fluid were both gradually absorbed.

Barsoum & Gaddum (1936) described a rise in blood histamine in human patients after burns, and this was confirmed by Code & Macdonald (1937), and by Rose & Browne (1940, 1942). The rise, however, does not occur at the same time as the shock. The significance of this phenomenon is thus obscure.

Various Japanese workers have studied this problem and the work of Kisima (1938) appears important, but is difficult to assess as the details are published in Japanese. He came to the conclusion that, when dog's skin was burned, there was a marked rise in the histamine content of the skin itself and also of the blood, urine, lungs, liver, kidney, spleen, pancreas, and intestine. He attributed these changes to the formation of histamine in the burned skin, and showed that, if the burned skin was removed, shock did not occur and the histamine content of the tissues did not rise. Lambert & Rosenthal (1943) have also come to the conclusion that burning causes the formation of histamine in dog's skin.

Methods

Mice were used in the experiments described here because it is comparatively easy to make an extract of the whole mouse and its excreta. It is thus possible to distinguish effects caused by the destruction or formation of histamine from those due to its transference from one part of the body to another. The technique was similar to that used by Alexander (1944) in this laboratory to study the disappearance of injected histamine. The mice were all male and white, weighing 20–30 g. and fed on oats, bread and bran. They were anaesthetized with ether and then plunged wholly, except for the head and neck, into hot water at 60° C. (thermostatically controlled) for 10 or 30 sec. Immediately after they had been removed from the water-bath they were carefully dried with filter paper. In some experiments they were killed within 10 min.; in others they were placed in metabolism cages in a warm room and given free access to water. The faeces and urine were collected together in the first two series of experiments. In the third series only urine was collected. After different periods they were killed by a blow on the head. Each carcass was divided into three fractions, the whole skin, the entire gastro-intestinal tract (with contents), and the rest of the body, and extracted by the methods described below. The excreta were either collected in trichloroacetic acid or preserved by the addition of chloroform to the collecting vessel.

Extraction of histamine. This was carried out in two distinct ways:

(*a*) *Chemical.* In some experiments, histamine was extracted by the method of Barsoum & Gaddum (1935) as modified by Code (1937). Minced tissues were ground in a mortar, and extracted for 1 hr. with 10% trichloroacetic acid, using 1·5 c.c. of acid to 1 g. tissue. Faeces and urine were treated likewise. After filtering by gravity, an aliquot of the filtrate was taken and heated with 10 c.c. of conc. HCl for 1·5 hr. on a boiling water-bath. The liquid was then evaporated to 5 c.c. *in vacuo* and the rest of the acid and water removed by washing the flask down

twice with absolute alcohol, and taking to dryness *in vacuo* each time. The rest of the procedure was as originally described.

(*b*) *Electrodialysis.* In some experiments the less drastic method of electrodialysis in a three-compartment cell, as described by MacGregor & Thorpe (1933), was used. A small cell, each compartment of which was of 15 c.c. capacity, with cellophane membranes separating the chambers, was assembled. The cathode was nickel sheet, and the anode was carbon. The cathode and anode compartments were filled with 10 or 15 c.c. of distilled water, and the middle compartment was filled with urine (0·2–3 c.c.) diluted with distilled water to 10 c.c., or with whole minced skin (4–7 g.) suspended in sufficient distilled water to give a final volume of 15 c.c. A current of 0·4 amp. was passed through the cell for 30 min. The temperature was kept below 40° C. by placing the apparatus in running water when necessary. The minced skin in the middle compartment was stirred with a glass rod from time to time to prevent any local aggregation of mince. The cathode liquid, water clear in the case of urine, and yellowish in the case of skin, was removed, and the cathode compartment washed out with distilled water. The combined liquid and washings were neutralized with 2N-HCl using Universal Indicator (B.D.H.), and then diluted, if necessary, to a suitable volume with Tyrode's solution in readiness for the biological assay. Removal of histamine was almost complete after 30 min. of electrodialysis, the content of histamine in extracts tested after 30, 40, 50 and 60 min. of dialysis being almost exactly the same. The active substance in the extracts was thermostable in N-HCl (boiling for 30 min.).

Estimation and identification of histamine. The extracts were first tested on a piece of guinea-pig's ileum suspended in 2 c.c. of Tyrode's solution containing atropine (0·1 μg./c.c.), in comparison with a standard solution of histamine phosphate. Concentrations are all calculated in terms of histamine base, on the assumption that this represents one-third of the weight of the phosphate.

This test is unspecific by itself. The conclusion that the active substance was histamine depends on the following facts:

(1) The extracts were so prepared as to include only substances soluble in trichloroacetic acid, and stable to boiling for 1·5 hr. in concentrated HCl. Histamine is the only known active substance in tissues with these properties. It was also shown that the active substance migrated towards the cathode through cellophane.

(2) In some cases, at the end of the experiment, a large excess (40–50 μg.) of histamine was added to the bath. After this treatment the preparation became temporarily insensitive to small doses of histamine, but remained sensitive to certain other stimulating substances such as carbachol. When the preparation was desensitized to histamine in this way it also became insensitive to the extracts.

(3) Most of the extracts, both before and after burning, were also tested in comparison with histamine by intravenous injection into atropinized cats anaesthetized with sodium pentobarbitone ('Veterinary Nembutal' given intravenously after induction with ether; 0·38 c.c./kg.). The depressor action of the extracts was indistinguishable from that of pure histamine phosphate, and the estimate of their histamine equivalent, obtained in this way, agreed in every case with that obtained by means of the guinea-pig's ileum with a maximum error of 25 %. This quantitative agreement provides strong evidence for identifying the active substance as histamine.

Body temperature. In some experiments the rectal temperature was measured with a thermocouple.

RESULTS

In the first series of experiments the conditions resembled those chosen by Leach, Peters & Rossiter (1943); a temperature of 60° C. was applied for 30 sec. This was found to kill most mice in 7–10 hr. with marked oedema of the subcutaneous tissue. The mice were almost motionless and paid no attention to their surroundings. They made no spontaneous movements, their limbs

appeared toneless, and they responded to stimuli with very brief and limited movements of the head. The rectal temperature was very low.

In the second series of experiments the time of exposure to 60° C. was reduced to 10 sec. in order that the later changes might be followed over a longer period. In each prolonged experiment of this second series there was included a control unburnt mouse. The histamine content of its tissues was invariably lower than that of the burnt mice. The average values for the control mice give an unbiased estimate of the normal values among these

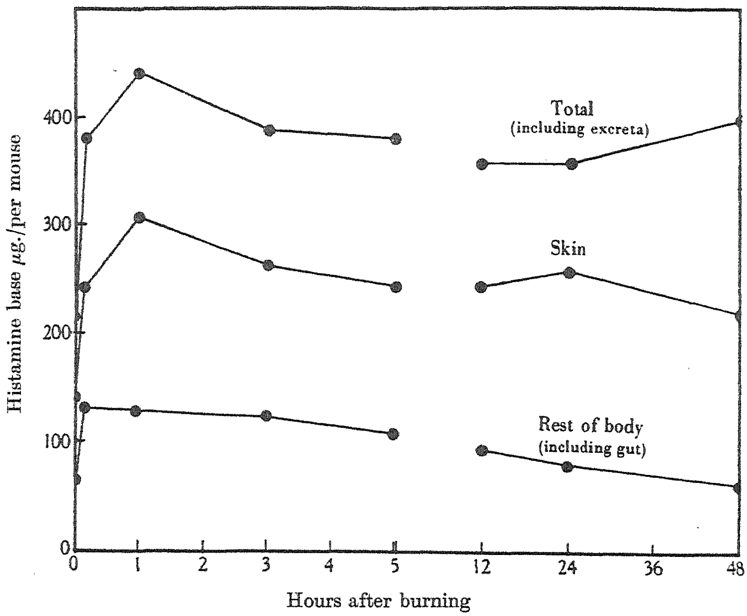

Fig. 1. Histamine content at different times after burning.

mice. In order to assess the factors involved, the control mice were anaesthetized and then plunged in water at room temperature. This did not appear to affect the histamine content. Heat, therefore, was the only known factor which could account for the differences in histamine content between the control mice and the experimental mice.

The individual results of these first and second series of experiments with extracts prepared chemically are given in Tables 1 and 2, from the average results of which Fig. 1 has been constructed.

The average total amount of histamine in the mouse's body rose during the first hour after the burn from 216 to 440 μg. There was no evidence of an increase in the histamine in the gut, but the proportional increase was about the same in the skin and in the rest of the body. Since 60–70% of the histamine in a mouse is in the skin, the total increase was mainly due to an

TABLE 1. Individual results of series 1. Histamine equivalents (μg.) for normal (N) mice and mice exposed to 60° C. for 30 sec. Extracts prepared chemically

Time after burning		Skin	Stomach and intestines	Rest of body	Excreta*	Total (including excreta when obtained)
	(N)	110	10	72	—	192
		146	11	81	—	238
		172	6	45	—	223
		137	13	62	—	212
1–4 min.		164	10	70	—	244
		164	8	72	—	244
		140	7	82	—	229
		205	7	61	—	273
5–10 min.		230	17	185	—	432
		282	14	91	—	387
		260	17	125	—	402
		223	11	113	—	347
		227	13	90	—	330
1 hr.		282	13	78	0·11	373
		294	15	154	—	463
		288	10	125	—	423
		378	9	115	0·08	502
3 hr.		253	11	88	—	352
		302	6	157	0·15	465
		264	18	125	—	407
		231	14	76	0·25	321
5 hr.		281	10	73	0·8	364
		225	3	182	0·2	410
		230	14	166	1·2	411
		233	16	100	1·1	350

* Faeces only, no urine excreted.
(N) = neither anaesthetized, nor immersed in water, nor burned.

TABLE 2. Individual results of series 2. Histamine equivalents (μg.) for control (C) mice and mice exposed to 60° C. for 10 sec. Extracts prepared chemically

Time after burning or immersion hr.		Skin	Stomach and intestines	Rest of body	Excreta	Total (including excreta)
5		242	7	72	—	321
		215	12	90	17	334
		305	8	78	0·9*	392
		215	13	85	75	388
12		267	10	86	2*	365
		245	9	105	2·8*	362
		230	12	74	20	336
		228	13	70	40	357
	(C)	*132*	*8*	*45*	*1*	*181*
24		264	7	62	20	353
		268	9	54	20	351
		281	6	75	1*	362
		220	8	95	40	363
	(C)	*110*	*5*	*35*	*1·3*	*157*
48		210	10	50	60	330
		176	6	50	100	332
		267	8	40	180	495
		226	8	60	144	437
	(C)	*146*	*5*	*34*	*3*	*188*

* Faeces only, no urine excreted.
(C) = anaesthetized and immersed in water at room temperature.

increase in the skin where the concentration rose from an average value of 24·4 to 47·8 µg./g. of wet tissue. A marked increase was observed within 10 min., and the maximum value was reached within an hour or less. The high concentration was maintained for 24 hr. There was some evidence of a fall after this time, but the concentration was still high after 48 hr.

Abnormal amounts of histamine were found in the first samples of urine collected after the burn, and the excretion of large amounts continued for at least 48 hr. The average amount recovered from the excreta in 48 hr. was 121 µg. per mouse, and it seems likely that this is the main route by which the excess histamine disappears from the body. There is, in fact, no evidence of any destruction of histamine in these mice. The figures for the total recovery of histamine from the mice and their excreta in the second series of experiments are remarkably constant between 5 and 48 hr. after the burn.

Electrodialysis. The first two series of experiments showed that the total amount of histamine extracted by a drastic process was increased by burning, and that the excess histamine was excreted in the urine. The histamine estimated in this way is, of course, not necessarily all present in an active form in the body. It may be largely formed from inactive precursors by the acid hydrolysis. It was also possible that the observed effects of burning were due to the liberation of preformed histamine from a compound which was not extracted by trichloroacetic acid. A third series of experiments was

TABLE 3. Histamine equivalents (µg.) for normal (*N*) mice, control (*C*) mice, and mice exposed to 60° C. for 30 sec. (skin experiments) and 10 sec. (urine experiments). Extracts obtained by electrodialysis. All tests on guinea-pig's ileum only, unless stated to the contrary

Time after burning		Skin µg.	Skin µg./g.	Time after burning or immersion hr.		Urine µg.	Urine µg./c.c.
	(*N*)	107·2	15·3		(*C*)	*0·12*	*0·24*
		62·9	15·7	12		13	65
		100	18·1			10·1	50·5
		80	17·7*			13	43·3
	Mean	87·1	16·7		Mean	12	52·9
5–10 min.		214·5	35·7		(*C*)	*0·067*	*0·08*
		214·4	33	24		21·3	42·6
		140·7	28·1			44·8	89·6
		147	29·5*			21·4	23·7
	Mean	179·1	32·1			39·2	39·2
1 hr.		187	28·8		Mean	31·7	39
		148	29·6		(*C*)	*0·26*	*0·13*
		127·3	32·3				
		133	33*	48		80	26·7
	Mean	148·8	31			60	30
						46·2	23·1
						150	50
					Mean	84	32·5

* Tested on cat's blood pressure.
(*N*) = neither anaesthetized, nor immersed in water, nor burned.
(*C*) = anaesthetized and immersed in water at room temperature.

therefore undertaken in which the most important parts of the earlier experiments were repeated, using electrodialysis to prepare the extracts. The results are recorded in Table 3.

The estimates of histamine obtained by electrodialysis were uniformly less than those obtained in the earlier experiments. This discrepancy may have been caused by the different method of extraction. The earlier estimates would include both free histamine and the combined histamine detected by Anrep, Ayadi, Barsoum, Smith & Talaat (1944). The extracts obtained by electrodialysis would presumably include only free histamine. Alternatively, the difference may be due to some unknown difference in the mice. The important point is that all experiments show that there is a large increase in the histamine

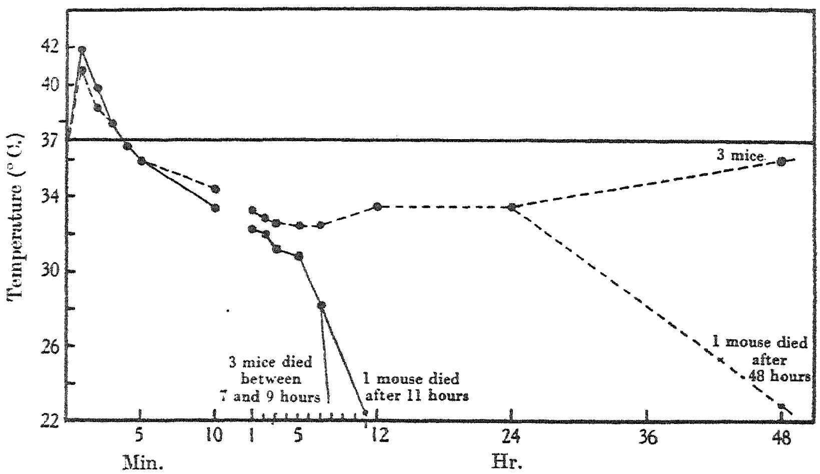

Fig. 2. Average rectal temperature in mice burned at 60° C. for 30 sec. (——) and for 10 sec. (-----).

content of the skin during the first hour after the burn, and that there is also an excess of histamine in the urine during the first 48 hr. The fact that these changes were detected by such a mild method of extraction as electrodialysis suggests that the increase is in the free histamine. On the other hand, if the apparent increase of histamine is due to the break up of a compound not extracted by trichloroacetic acid, this hypothetical compound must also fail to be carried through cellophane to the cathode. There is no reason to suggest the existence of such a compound.

Body temperature. Measurements were made of the body temperature, partly to determine whether the observed changes should be attributed to a local burning of the skin or to a rise in the general temperature of the body, and partly as a means of assessing the clinical condition of the mice. The results are shown graphically in Fig. 2, in which each point, unless shown to the contrary, represents the mean of four observations.

The mean rectal temperature rose during burning from a normal value of 37° C. to 40·8° after exposure for 10 sec., and to 41·8° C. after exposure for 30 sec. At the end of 10 min. it had fallen to about 34° C. When it fell below 32° C. the mice invariably died. In the mice which recovered, the temperature began to rise as the histamine disappeared on the second day.

It is unlikely that the slight hyperthermia shown by these results was the cause of the observed formation of histamine, though this possibility cannot be definitely excluded. It is more likely that the effect was due to the local burning of the skin. The ensuing hypothermia was presumably due to shock, and showed that this occurred at the same time that there was an increased amount of histamine in the mouse.

Discussion

The results show that histamine was formed in the skin within 10 min. of the application of heat, and this confirms the results of Kisima (1938) and Lambert & Rosenthal (1943), but not those of Harris (1927). The reasons for this discrepancy are under investigation.

This formation of histamine is particularly interesting because other forms of injury, such as anaphylaxis (Feldberg, 1941), are associated with the liberation of preformed histamine rather than with the formation of new histamine. It seems likely that this phenomenon plays some part in the circulatory response of the body to burns, but its full significance is not yet clear. The mice used in these experiments were all severely injured, but it is improbable that any significant part of the injury was due to the action of histamine which had been absorbed from the skin into the general circulation. The quantity formed was too small to have much effect on mice, which are remarkably insensitive to histamine, and appear to be scarcely inconvenienced by the intravenous injection of quantities 10 times larger. In other species, which are more sensitive to histamine, the absorption of this substance from burned tissues may have an effect, but it cannot have much effect on a mouse.

On the other hand, it is possible that the new formation of histamine in the skin played a significant part in the local response to injury, causing dilatation and increased permeability of the capillaries and dilatation of the arterioles by axon reflexes, and the consequent local accumulation of plasma at the site of the burn. It was observed that, within 1 hr., the subcutaneous tissues of these mice were distended with oedematous fluid which was liable to set into a jelly even during life. This may have been due to the escape of plasma through capillaries whose permeability had been increased by histamine. This local and prolonged action of the histamine may be more dangerous than the alarming but short action of histamine injected intravenously.

Rose & Browne (1940, 1942) studied clinically the blood histamine in burn shock and reported that although it was increased immediately after burning

it was actually decreased in active shock as compared with control values and those after recovery. In patients who die. the blood histamine is very low just before death. This may perhaps be because in shocked patients the rate of histamine absorption from the tissues is decreased and may even cease during severe circulatory collapse, as is known to be the case with other drugs injected subcutaneously.

Until recently, it has generally been assumed that histamine is not excreted in appreciable quantities in the urine. Recent work has shown that this assumption was untrue (Kapeller-Adler, 1941; Alexander, 1944). The evidence has been reviewed by Anrep *et al.* (1944), who obtained evidence that the normal urine usually contains some histamine and that this may be free, or combined in a form which is pharmacologically inactive. It is probable that both forms of histamine were estimated together by the method used in most of the present experiments.

The traces of histamine estimated in normal urine might be due to experimental error, as it is difficult to separate urine and faeces completely, and to avoid contamination with desquamated epithelium. There can, however, be no doubt that histamine was excreted in the urine of burned animals. The fact that all the excess histamine was excreted, and that little or none of it was destroyed in the mouse, is interesting, but cannot usefully be discussed until more is known about the fate of histamine injected in comparable doses in mice. The data of Alexander (1944) were obtained with much larger doses (3 mg. per mouse).

Summary

1. Extensive cutaneous burns in mice caused the new formation of histamine, mainly in the skin, so that the total amount of histamine in the mouse was almost doubled in 10 min.

2. This excess histamine was mostly excreted in the urine during the next 48 hr., if the mice survived.

3. The relation of this phenomenon to shock following burns is discussed.

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ON THE VASODILATATION IN HUMAN SKELETAL MUSCLE DURING POST-HAEMORRHAGIC FAINTING

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It is generally agreed that when a person faints the sudden fall of the arterial blood pressure causes anaemia of the higher centres. Foster's (1888) explanation of the blood-pressure fall was that the vagus excited such an intense bradycardia that cardiac output and blood pressure suffered a decrease. The emphasis was laid on the failure of the heart; it was a cardiac syncope. Lewis (1932), however, observed cases of fainting without much bradycardia. Moreover, when atropine was injected into a subject just after he had fainted, the heart rate increased to normal but the blood pressure did not. Lewis introduced the term 'vasovagal syndrome' to show that blood vessels and heart were both implicated. He thought that the peripheral vasodilatation was the primary cause of the fall of the arterial blood pressure. This view was supported by the experiments of Barcroft, Edholm, McMichael & Sharpey-Schafer (1944), who observed no fall in cardiac output in man during fainting induced by venesection, but vasodilatation in the forearm.

They considered that (1) the dilatation was probably in the forearm muscles, (2) it was of nervous origin, and (3) if a similar dilatation took place throughout the whole skeletal musculature it would certainly cause a drop in blood pressure, which would be perhaps great enough to explain the fall observed during fainting.

The object of this paper is to give a more detailed account of the changes in the peripheral circulation in fainting in so far as they can be inferred from experiments on the blood flow through the forearm and hand. Observations have been made on normal subjects, on sympathectomized subjects and on subjects with the nerve supply to the forearm blocked.

Methods

Healthy men aged 20–30 acted as subjects. Room temperature was about 20° C.

The subject stripped to the waist. The left forearm was shaved. The plethysmograph for measuring blood flow by Lewis & Grant's method (Barcroft & Edholm, 1943) was fitted as shown in Fig. 1. The subject lay down in a comfortable position on a couch with his back raised to an angle

of about 45°. It is easier to induce fainting in this position than with the subject supine. The collecting cuff and wrist cuff were put on. The arm in the plethysmograph was placed in a comfortable position in an electrically heated and stirred constant temperature water-bath, with the water level just above the elbow, and the water temperature about 34° C. (Barcroft & Edholm, 1943). The subject was covered with blankets. The arrangements for recording the flow were completed, and the apparatus calibrated. A sphygmomanometer cuff was put on the right arm. A 6½ in. sphygmomanometer cuff was put on each thigh as high up the thigh as possible.

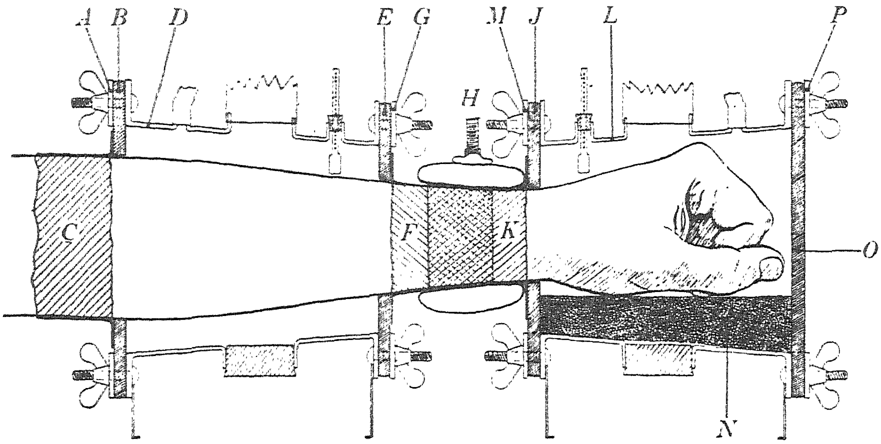

Fig. 1. To record the blood flow in the forearm the plethysmograph was fitted as follows. The brass ring *A* was slid up the arm, followed by the nicely fitting 'made to measure' rubber diaphragm *B* with its 2 in. long thin rubber cuff *C*. The cuff *C* was rubber cemented to the skin as high up the forearm as possible. Plate *A* and diaphragm *B* were then bolted to the plethysmograph *D*. Diaphragm *E* and attached cuff *F* were then slid into position, and the cuff *F* cemented to the skin. Diaphragm *E* was plated to the plethysmograph with two semicircular plates *G*.

To record the blood flow in the hand the plethysmograph *L* was fitted as follows. Cuff *H*, made of cycle inner tubing, was slipped on the forearm. Next came diaphragm *J* with attached cuff *K*. The position of the diaphragm was adjusted so that it was just over the wrist joint. Cuff *K* was cemented to the skin. The plethysmograph *L* was of the same pattern as *D*. The opening in its narrower end was plated to the diaphragm *J* by plates *M* so that the hand was palm downwards. The flexed fingers were supported on a perforated zinc platform *N*. The wide end of the plethysmograph *L* was closed by a diaphragm *O* plated on by ring *P*. Finally the wrist cuff *H* was slid distally over cuff *K* till quite close to the plethysmograph, where it was covered with insulating tape to prevent it ballooning outwards. To record the blood flow in the forearm and in the hand simultaneously the forearm plethysmograph and then the hand plethysmograph were fitted as above. With a little manipulation cuff *K* was cemented to the skin and to cuff *F* under the venous occlusion cuff *H*.

One observer was responsible for recording blood flow, blood pressure (B.P.) and pulse in the left arm; a second for B.P., pulse, and venesection in the right arm; a third for inflation of the thigh cuffs and for noting symptoms, etc.

Blood pressure, blood flow and pulse rate were recorded at 5 min. intervals in the first part of the experiment.

After three or four readings the cuffs on the thighs were inflated with air to 80 mm. Hg, and, during the next 20 min., the pressure was gradually raised to about 10 mm. below systolic pressure.

This procedure dams back blood in the veins of the legs and so produces the equivalent of a large haemorrhage (McMichael & Sharpey-Schafer, 1944; Ebert & Stead, 1940). 30 min. after inflating the thigh cuffs B.P. measurements on the right arm ceased and venesection began. No local anaesthetic was used. An arbitrary maximum of 5 c.c. blood per lb. body weight was fixed for the volume of blood to be drawn off, and it required 10–15 min. to remove the full quota. If the subject fainted before this quota had been withdrawn, the needle was removed and venesection ceased. After venesection, the thigh cuffs were kept inflated till (a) the subject fainted, (b) a gradual rise of the B.P. showed that he was unlikely to faint, or (c) the legs had been congested for 1 hr. (The above technique induced fainting in about 90 % of the subjects.)

Until the onset of fainting, the collecting pressure on the plethysmograph arm was adjusted in accordance with the B.P., and was kept at approximately diastolic pressure. During fainting, when the B.P. falls precipitously, it is essential to use a very low collecting pressure, i.e. 30 mm. Hg or less, otherwise the usual collecting pressure may be well over the level of the systolic pressure and no blood flow will be recorded. As soon as fainting began the collecting pressure was quickly lowered to about 30 mm. Hg. Blood flows were recorded at about 1 min. intervals. The wrist cuff was kept inflated between readings. B.P. readings were made at short intervals. In the fully developed faint the B.P. was sometimes unobtainable. The pulse was generally impalpable. The heart beat could often, but not always, be auscultated at the apex.

After two flow-records had been taken in the fully developed faint the pressure in the thigh cuffs was released, so that the blood dammed back in the lower limbs was returned to the general circulation. If the faint was severe the head was lowered. B.P. usually began to recover within 3 min. (In two cases in which it did not, an intramuscular injection of 20 mg. methedrine was given and the blood pressure rose rapidly. During the war, in this country alone, more than 25,000 blood donors have fainted and all have recovered.)

Recording was continued during recovery. If more than 500 c.c. of blood was withdrawn, the subject was transfused. Recording stopped $\frac{1}{2}$–$\frac{3}{4}$ hr. after the faint, or rather later if transfusion had been done. By the time the plethysmograph had been taken off, the subject felt perfectly fit to go on with his occupation. During the recovery period the subject was asked to recall any symptoms noticed before fainting. One 10-stone subject who lost 700 c.c. and was not transfused had a second delayed faint. Others felt a little tired; no other after-effect was ever noticed.

For measuring hand flow, Freeman's (1935) method was used. The hand plethysmograph was fitted as shown in Fig. 1.

In two experiments, forearm and hand flows were measured simultaneously in the same limb (see Fig. 1). In these experiments two schemes for taking the records were tried. In one, turning a single tap threw the collecting pressure into the cuffs on the upper arm and on the wrist simultaneously. In the other, the blood flow through the hand was recorded first, then, after an interval of 1 min. the circulation in the hand was arrested and the forearm flow taken. Owing to the greater initial distortion when flows were measured simultaneously, independent recordings were preferred.

The sympathectomized forearm. Preliminary tests were made to see if the sympathectomy had been complete. Two tests were used: Landis & Gibbon's (1933) body heating and finger temperature test; and the body heating and forearm blood flow test (Prinzmetal & Wilson, 1936; Wilkins & Eichna, 1941). Both tests are based on the fact that rise in the body temperature causes a vasodilatation in the arm, mediated by the sympathetic. A limb was regarded as completely sympathectomized when the normal rise in finger temperature and forearm blood flow were absent. These tests enabled us to exclude one subject who did not conform to these standards.

In this group of six subjects there were three females, and the age limits of 16–64 were wider than in the normal group. It is unlikely that these factors could have affected the significance of the results.

The nerve-block forearm. The musculo-spiral, median and ulnar nerves in the left forearm were each injected with 2 c.c. of 4 % procaine in 1 in 50,000 adrenaline (Barcroft, Bonnar, Edholm & Effron, 1943). After 15 min. the injections were repeated, and by this time, in almost all cases, the movements of the hand were rapidly becoming paralysed. In a few subjects, in whom the block

was unsatisfactory, the experiment was given up at this point. As soon as the injections had been repeated, the thigh cuffs were inflated. The arrangements for recording the forearm blood flow were completed. One or two estimations of pulse, B.P. and forearm flow were made. The hand was examined to check the paralysis. Half an hour after the thigh cuffs were inflated, venesection was begun.

RESULTS

Blood flow in the forearm during fainting. Results were recorded in nine faints. Typical data are shown in Fig. 2. The time-span in this and all subsequent diagrams is 50 min., 25 before and 25 after the deepest point of the

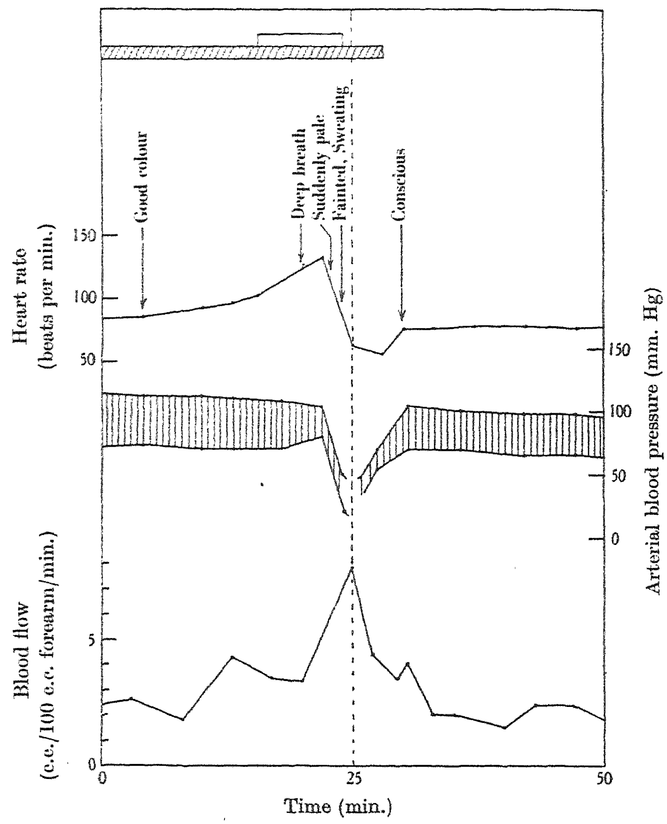

Fig. 2. Post-haemorrhagic fainting. Typical symptoms and typical changes in the heart rate, in the arterial blood pressure and in the blood flow in the *normal forearm.* Plain rectangle: venesection. Shaded rectangle: venous tourniquets on both thighs.

faint as judged from the B.P. and subject's condition. In all diagrams the 25th min. is marked by a vertical broken line; the shaded rectangle shows the duration of venous congestion of the legs, and the plain rectangle shows the duration of the venesection. The symptomatology of post-haemorrhagic fainting has been described by Wallace & Sharpey-Schafer (1941) and by

Brown & McCormack (1942). Our observations on the signs and symptoms will be described elsewhere. The top curve in Fig. 2 shows the typical bradycardia. The B.P. record shows the acute fall from about 105/90 to about 50/20 mm. Hg. In this experiment the subject fainted during the venesection which was stopped before the full quota, 770 c.c., had been withdrawn. Recovery began before the pressure in the thigh cuffs was released. The behaviour of the forearm blood flow is shown at the bottom of the diagram. During the faint, forearm blood flow rose from 3·3 to 6·8 c.c. per 100 c.c. forearm per min., in spite of the acute fall of B.P. There must therefore have been a very marked dilatation of the vessels in the forearm to bring about a doubling of the rate of the blood flow when the B.P. was falling so fast. Fig. 3 shows a typical tracing.

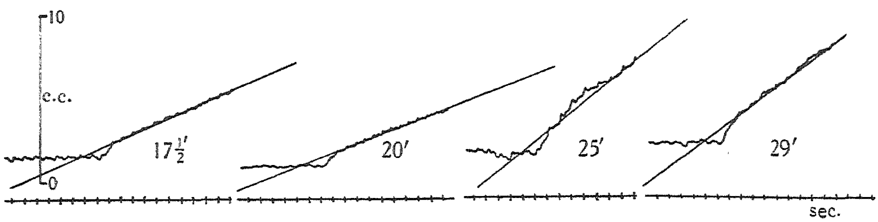

Fig. 3. Tracings of the blood flow in the *normal forearm* obtained by Lewis & Grant's plethysmographic method in the experiment shown in Fig. 4, No. 1. Taken at the times marked on the tracing. Fainting began soon after 20 min., and was fully developed at 25 min.

The B.P. and forearm blood flows of all nine experiments in this group are shown in Fig. 4. Every experiment except No. 5 shows increase in the forearm flow during the acute B.P. fall of the faint—proof of vasodilatation. The faint in No. 5 was severe, breathing was stertorous, the pulse slowed to 30 beats per min. and the B.P. was unobtainable for some minutes. Yet in spite of this profound drop in the B.P. the forearm flow did not decrease, so here too there must have been vasodilatation.

The averages of the B.P. and forearm flows of the group are shown in Fig. 5. There is thus no doubt that when a person faints from blood loss a peripheral vasodilatation in the forearm occurs.

Blood flow in the hand during fainting. Barcroft *et al.* (1944) had two reasons for believing that the vessels which dilated were in the forearm muscles. Firstly the death-like paleness of the skin of the face seemed incompatible with increase in the rate of the circulation through the skin. Secondly Weiss, Wilkins, & Haynes (1937) had found a decrease in the rate of the blood flow through the hand during fainting. Apparently, in fainting, hand and forearm blood flows alter in opposite directions. This could be explained by supposing that there is hyperaemia in muscle and ischaemia in skin and bone. The hand is about 55 % skin and bone (Abramson & Ferris, 1940) and has only 15 % muscle so

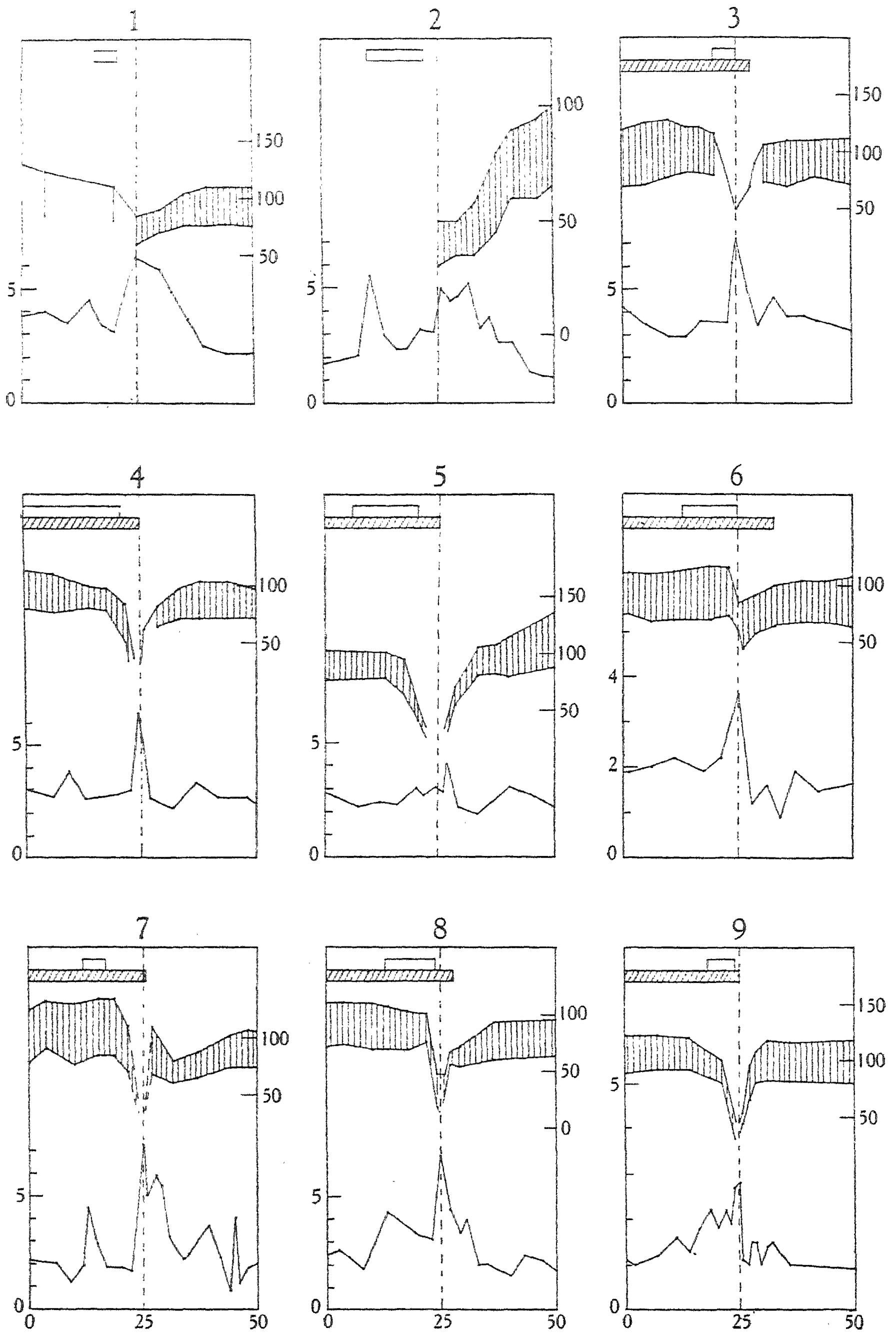

Fig. 4. Plain rectangle: venesection. Shaded rectangle: venous tourniquets on both thighs. Upper curve: arterial blood pressure, mm. Hg. Lower curve: blood flow in the *normal forearm*, c.c./100 c.c. forearm/min. Time in min. Broken vertical line: faint fully developed. In the experiment shown in No. 6, 20 mg. methedrine was given intramuscularly at 28½ min.

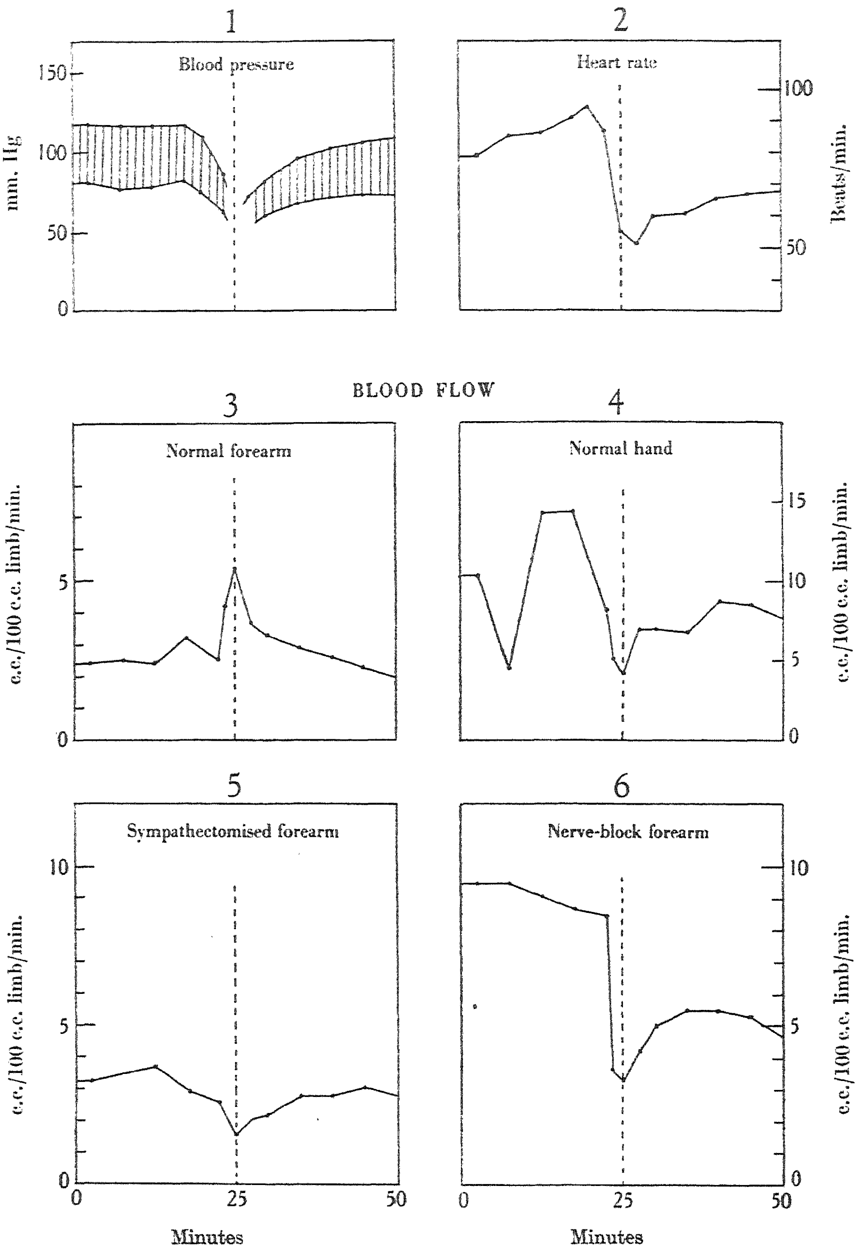

Fig. 5. Averages of results of experiments. Broken vertical line: faint fully developed.

the net effect would be ischaemia. On the other hand the forearm is 60 % muscle, with only 20 % skin and bone, so the net effect would be hyperaemia.

There was one important point about the validity of the results obtained by Weiss *et al.* The hand flows which they recorded during fainting were, to all intents and purposes, nil. This result would have been obtained if they used

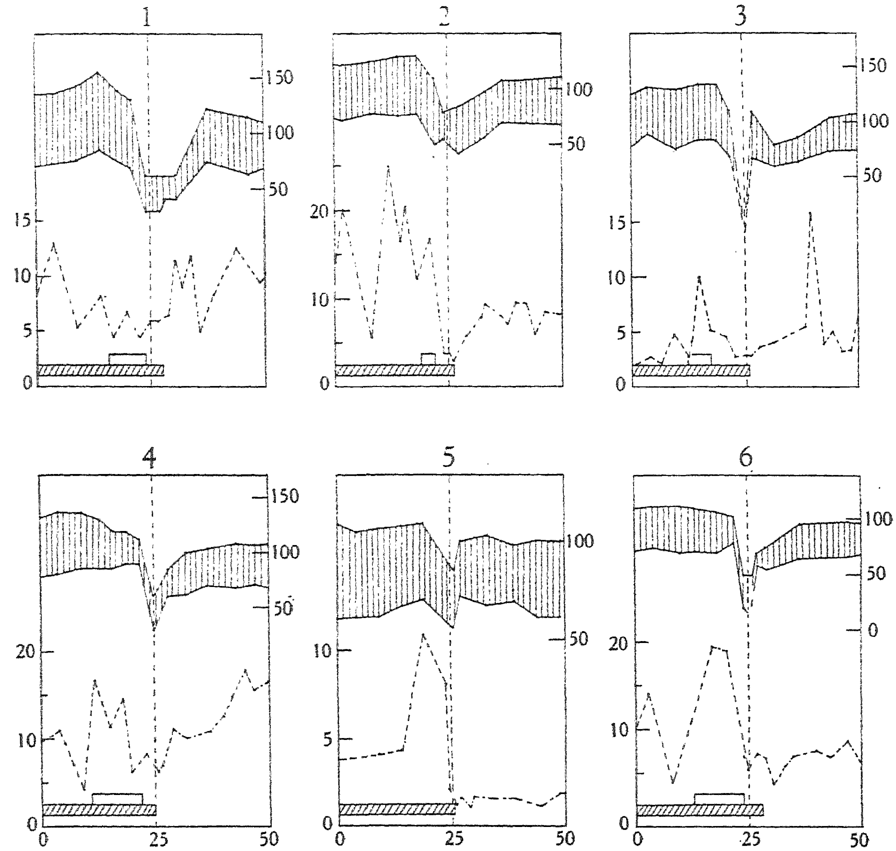

Fig. 6. Upper curve: arterial blood pressure mm. Hg. Lower curve: blood flow in the *normal hand*, c.c./100 c.c. hand/min. Plain rectangle: venesection. Shaded rectangle: venous tourniquets on both thighs. Time in min. Broken vertical line: faint fully developed.

the usual collecting pressure of about 60–70 mm. Hg and did not take the precaution to lower it during the faint; after the collapse of the blood pressure, 60–70 mm. Hg might have arrested the circulation in the hand. Also they induced fainting by administration of amyl nitrite, followed by tilting the subject to the upright position, so that possibly the mechanism of post-haemorrhagic fainting was different. These considerations led us to examine the hand flow in post-haemorrhagic fainting.

In the normal person, hand flows are much greater than, and fluctuate much more than, forearm flows (Abramson & Ferris, 1940). The B.P. and hand flows of six faints are shown in Fig. 6. The consistent thing about them is the low hand flow shown at the onset of the faint in every experiment. The flows in

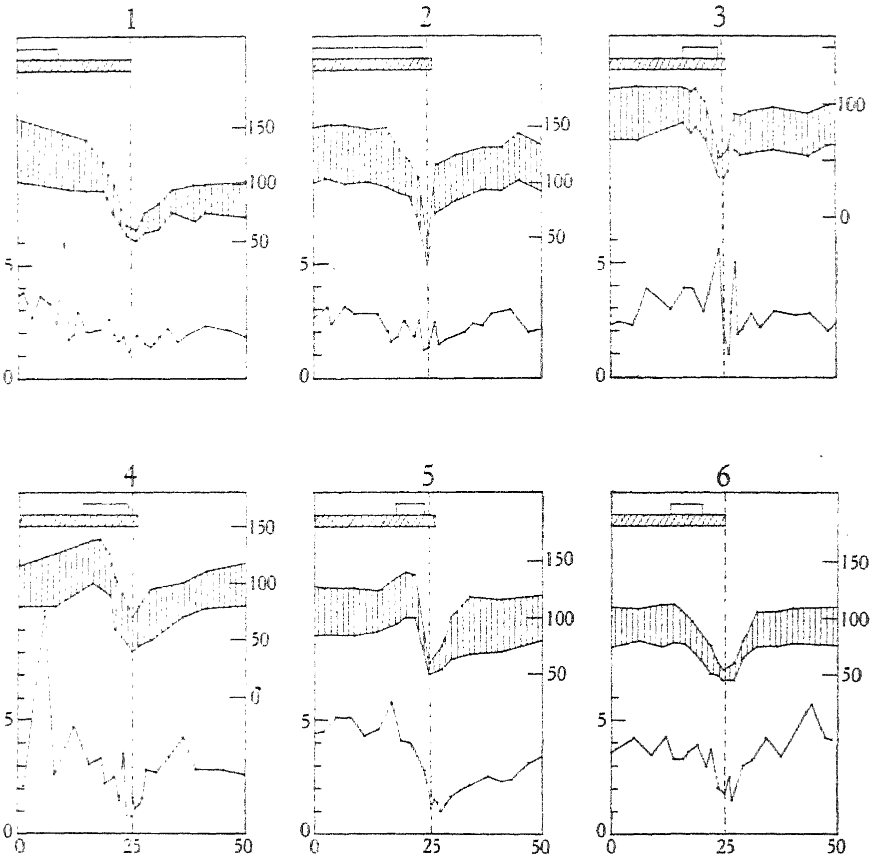

Fig. 7. Plain rectangle: venesection. Shaded rectangle: venous tourniquets on both thighs. Upper curve: arterial blood pressure in mm. Hg. Lower curve: blood flow in the *sympathectomized forearm* c.c./100 c.c. forearm/min. Time in min. Broken vertical line: faint fully developed.

Nos. 3 and 6 were recorded simultaneously with the forearm flows of Fig. 4, Nos. 7 and 8. In both experiments we see small hand and large forearm flows during fainting.

The hand flows for the group have been averaged and the results plotted in Fig. 5, No. 4. There is no doubt about the decrease in the blood flow through skin and bone in fainting. The increase in the forearm flow must have been in the muscle.

Blood flow in the sympathectomized forearm during fainting. To decide the mechanism of the vasodilatation in muscle during fainting, experiments were carried out on subjects with upper limb sympathectomies. If the dilatation were brought about by the vasomotor centre via the sympathetic, it should be absent after sympathectomy. If any other factor were responsible, the dilatation should still be obtained.

The B.P. and forearm flows of six sympathectomized subjects are shown in Fig. 7. The results are strikingly different from those on the normal forearm (Fig. 4), there is no suggestion of an increase in the flow during fainting. The flow is at a minimum at, or close to, the broken vertical line. At first sight No. 3 seems to gainsay the others. As the B.P. starts falling steeply at the beginning of fainting, forearm blood flow increases, just as in the normal forearm. Then it falls to a low level at the broken line, then rises to a peak again as recovery occurs. We think the explanation may be as follows. Sudden brief vasodilatations are seen in Fig. 7, no. 1 at about 10 min., in Fig. 7, no. 4 at about 5 min., in Fig. 4, no. 2 at about 10 min., and in Fig. 4, no. 7 at about 15 min. 'Spontaneous' vasodilatations like these are absent when the subject is mentally at rest. They are almost certainly emotional reactions, due to the stress of the experiment. Similar reactions have been described by Wilkins & Eichna (1941), who thought they were caused by adrenaline because they could be elicited in sympathectomized but not in adrenalectomized subjects. The faint in Fig. 7, no. 3, may coincide with a strong emotional dilatation, and so the forearm blood flow is atypical. For that reason this experiment was omitted from the averaged forearm flows of this group which are seen in Fig. 5, no. 5. The figure shows that sympathectomy abolishes the increase in the forearm blood flow which takes place in the normal subject in fainting.

Blood flow in the nerve-block forearm during fainting. The vasodilatation observed in the forearm muscles of the normal person during fainting might be due to release of vasoconstrictor tone or to active vasodilatation. This might be decided by comparing the average blood flow during fainting in the normal and sympathectomized forearm. If it were greater in the former, active vasodilatation would be involved. However, it is not advisable for this purpose to use the results obtained with the sympathectomized subjects described above, since within a few weeks of sympathectomy the immediate post-operative increase in blood flow has practically disappeared (Grant & Holling, 1938). But such sympathectomies can be carried out temporarily by means of a nerve block of the median, ulnar and radial nerves (Barcroft *et al.* 1943). There is no appreciable spontaneous recovery of tone in the short interval between the block and the induction of fainting (Barcroft & Edholm, 1944). Blocking the motor and sensory fibres is unavoidable, but Barcroft *et al.* (1943) have shown that this has no effect on the blood flow.

Fig. 8 shows data from six nerve-block subjects. Comparing Fig. 8 with Fig. 4 one sees that the initial level of flow in the nerve-block forearm is more than double that in the normal forearm (Barcroft *et al.* 1943). This is because the vasoconstrictor fibres were paralysed and tone in the muscle blood vessels

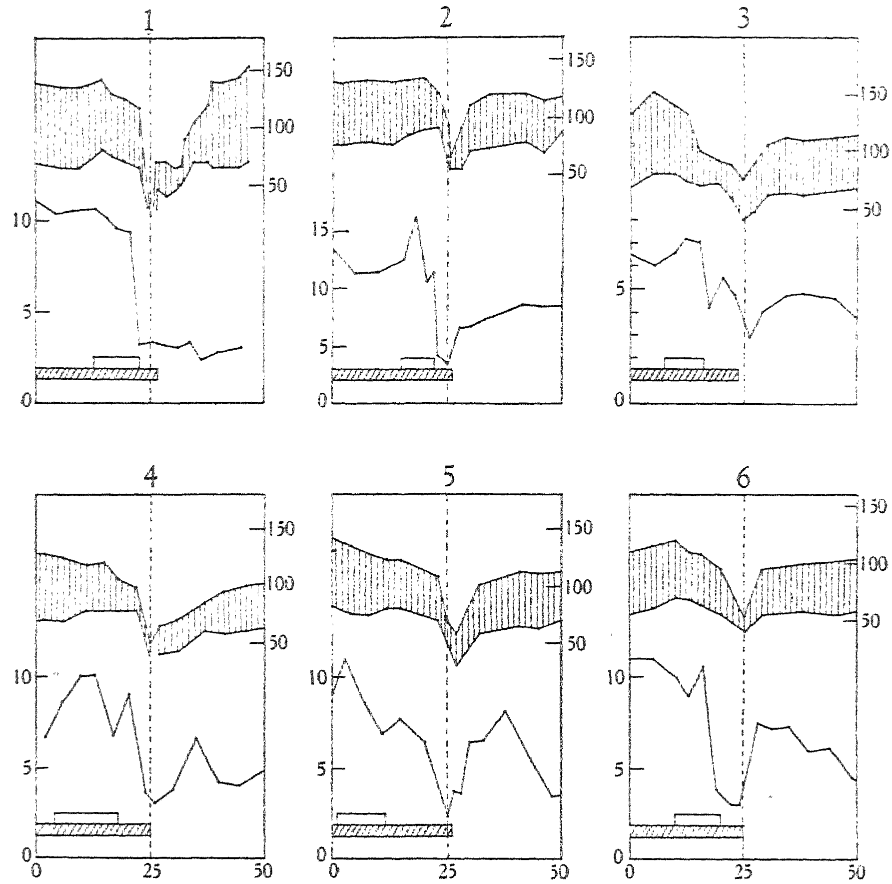

Fig. 8. Upper curve: arterial blood pressure in mm. Hg. Lower curve: blood flow in the *nerve-block forearm*, c.c./100 c.c. forearm/min. Plain rectangle: venesection. Shaded rectangle: venous tourniquets on both thighs. Time in min. Broken vertical line: fully developed faint. In the experiment shown in No. 1, 30 mg. methedrine was given intramuscularly at 28 min.

abolished. In Fig. 8, B.P. and blood flow fall together in every experiment. B.P. recovers as the faint passes off, so to a less extent does blood flow; recovery of the blood flow may be complicated by an injection of methedrine in one experiment, Fig. 8, no. 1, and by the effect of the local anaesthetic beginning to wear off in the other experiments. Fig. 5, no. 6, shows the average forearm flow for the group. The features are those just described. The average

blood flow through the acutely 'sympathectomized' forearm during fainting is seen to be 3·3 c.c. Normal forearm blood flow was much greater during fainting—average figure 5·3 c.c.—implying that the vasomotor centre stimulated active vasodilatation via the sympathetic.

Discussion

The vasodilatation in the forearm during post-haemorrhagic fainting, reported by Barcroft *et al.* (1944) has been confirmed. Their suggestion that it was in the muscle has been investigated and verified. In addition, it has now been shown that the dilatation is actively excited by the vasomotor centre via sympathetic fibres. Sympathetic vasodilator nerves supplying the blood vessels of skeletal muscle have not previously been described in man. Bülbring & Burn (1937) have demonstrated the presence of such fibres in dogs and in hares. The blood vessels of skeletal muscle are therefore supplied by both constrictor and dilator fibres. There is no definite evidence as to the cholinergic or adrenergic character of the fibres.

The absence of the dilatation in the sympathectomized subjects excluded each of the following possible causes:

(1) Adrenaline. There is good evidence that this may be secreted in animals in haemorrhage (Tournade & Chabrol, 1925; Saito, 1928; Saito, Kamei & Tachi, 1928; Brooks, 1935) and that it can cause vasodilatation in human muscle (Grant & Pearson, 1938).

(2) Vasodilator impulses via the posterior roots.

(3) Loss of muscle tone in fainting. It was conceivable that the blood flowed more quickly through the relaxed muscle.

Grant & Pearson (1938) and Wilkins & Eichna (1941) studied the blood flow in the forearm and in the calf. The responses produced by a number of procedures were substantially the same in both parts. Both are mainly muscle. Therefore the behaviour of the circulation in the forearm muscles is probably a reliable index of what is happening elsewhere in skeletal muscle. If this is conceded, it follows that, during fainting, vasodilatation takes place in all skeletal muscles.

Barcroft *et al.* (1944) considered what effect this dilatation would have on the blood pressure. Their observations indicated that the state of the circulation at each of three different stages of a typical experiment was probably approximately as seen in Table 1.

The circulation through the skeletal muscles was estimated from the forearm blood flow, that through the remaining tissues was obtained by difference between cardiac output and skeletal muscle flow.

Haemorrhage caused a small fall in the B.P. and a large decrease in cardiac output. The B.P. must have been maintained, to a considerable extent, by

TABLE 1

	Arterial blood pressure mm. Hg	Mean blood pressure mm. Hg	Cardiac output c.c. min.	Skeletal muscle blood flow c.c. min.	Blood flow through all other organs c.c. min.
Before haemorrhage	130/70	100	5000	800	4200
After haemorrhage before fainting	105/75	90	3000	900	2100
Fully developed faint	50/30	40	3000	2000	1000

vasoconstriction of the non-muscular tissues. Gesell (1920) demonstrated decrease in blood flow through the salivary glands of animals during haemorrhage, and Gregersen (1941) has shown that in man a haemorrhage of 500 c.c. will only cause a slight fall of B.P. and reduce the salivary secretion to one-twentieth of the previous level.

The sudden and profound fall in the B.P. in the faint was not accompanied by any further decrease in cardiac output and therefore was not directly due to the loss of blood. It must have been caused by peripheral vasodilatation. There was evidence of such dilatation in the skeletal muscles since muscle flow was approximately doubled in spite of the profound fall in the B.P. Outside of the skeletal muscles, in the faint, blood flow must have decreased from about 2 to about 1 l. per min. This remarkable diminution accompanied the sudden reduction of the mean B.P. to about half its value immediately preceding the faint. The fact that both B.P. and flow decreased to about the same extent suggests that the reduction in flow was mainly a passive change due to the fall in the B.P., for Wiggers & Werle (1942) consider that, in passive changes, B.P. and flow are almost directly proportional. It may be concluded that vasodilatation in skeletal muscle was the prime cause of the acute fall in the B.P. during fainting.

Fig. 5, nos. 1 and 3 show that the time relations of the changes in B.P. and forearm flow during fainting and recovery are in fair accord with the idea that B.P. and muscle flow are inversely related.

The view that, apart from skeletal muscle, the peripheral vascular system behaves passively in post-haemorrhagic fainting needs further investigation. The loss of consciousness is generally attributed to passive decrease in the cerebral flow. The paling of the skin as the faint develops is probably mainly a passive effect since sympathectomized skin pales like normal skin. This was noticed in the experiments on the sympathectomized subjects. The decrease in the blood flow through the hand may have been due to the fall in B.P. Furthermore the intestines look pale during fainting. This was observed by Bailie (1944) in abdominal operations under spinal anaesthesia.

After injury and haemorrhage, it is likely that the vaso-vagal syndrome supervenes and the condition is probably comparable with that known as primary shock. In our experiments, recovery occurred very rapidly even in

cases where the blood lost was not replaced. The pallor and slow pulse however persisted. The pulse rate was not maintained at the slowest recorded rate but did not return to the fast rate found immediately before fainting supervened. Clinically this may be of some importance in that a subject who had lost sufficient blood, and fainted, might only be seen after recovery from the faint, when the pulse rate would be slower than expected. This might lead to confusion.

Haemorrhage is only one of many conditions that may excite the vaso-vagal syndrome. Others are:

(1) Sudden strong emotions (Lewis, 1932).

(2) Severe pain (probably) (Lewis, 1932).

(3) The maintenance of the upright position: (*a*) with the weight off the feet (Mayerson & Burch, 1939); (*b*) after administration of sodium nitrite (Weiss *et al.* 1937); (*c*) in rarefied air (Mateeff & Schwarz, 1935); (*d*) after strenuous exercise (Mateeff, 1935).

(4) Anoxia (Bauer, 1926).

(5) Spinal anaesthesia (Bailie, 1944).

In each of the circumstances enumerated above, the subject is liable to a sudden fainting attack characterized by bradycardia, pallor, sweating, unconsciousness and low B.P. Valuable information could be obtained from examination of the peripheral circulation in these faints.

It is interesting to speculate about the possible function of this mechanism, excited as it is by such varying causes. Is the subject after recovery from fainting in the same condition as before he fainted? Is the vago-vagal syndrome rightly called a circulatory collapse, or is it part of a protective mechanism which leaves the subject's circulation more stable than before?

Summary

1. Post-haemorrhagic fainting was induced by venous tourniquets on the thighs combined with venesection. This procedure induced fainting in twenty-eight out of thirty-two subjects.

2. Blood flow was studied in the normal forearm and hand, in the sympathectomized, and in the nerve-block forearm.

During fainting:

1. Blood flow in the normal forearm increases as the arterial pressure falls. Therefore there is vasodilatation in this region.

2. Blood flow through the hand, which is mostly skin and bone, decreases. Therefore the vasodilatation in the forearm is in the skeletal muscles.

3. Sympathectomy abolishes the forearm vasodilatation. Therefore it is brought about by the vasomotor centre and sympathetic nervous system.

4. Blood flow is greater in the normal than in the nerve-block, i.e. acutely sympathectomized, forearm during fainting. Therefore the vasodilatation is

actively excited; and there must be sympathetic vasodilator fibres in the forearm muscles.

5. The vasomotor centre probably excites vasodilatation in the arterioles of all skeletal muscles. This may explain the sudden fall in the arterial blood pressure in fainting.

The first three experiments on post-haemorrhagic fainting were performed in conjunction with McMichael & Sharpey-Schafer.

We express our deep appreciation of the help given by the members of the Belfast Medical Students' Association, by certain Naval Ratings, and by the sympathectomized subjects.

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RESTING AND ACTION POTENTIALS IN SINGLE NERVE FIBRES

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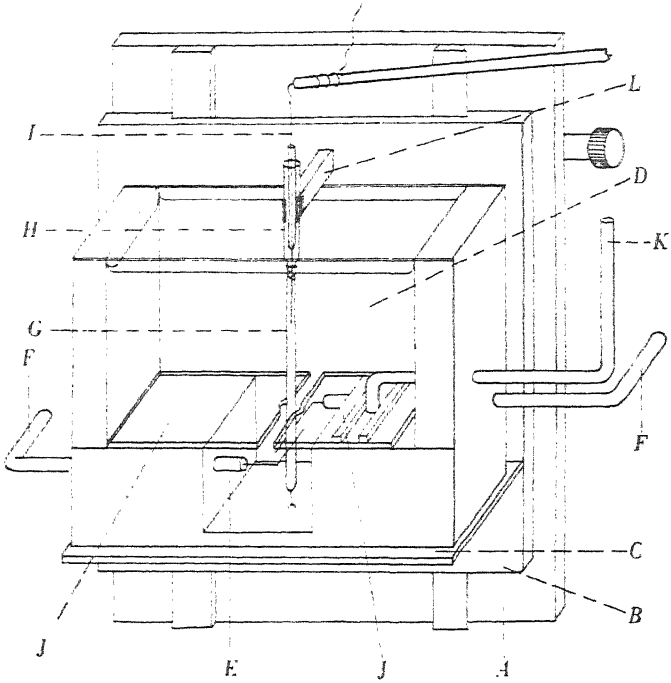
Until recently the action potential of nerve and muscle has been thought to arise from a transient breakdown of the polarized cell membrane. This hypothesis was widely accepted as an integral part of the membrane theory of nervous activity, although it rested upon little more than a rough equality in the apparent magnitudes of action and resting potentials. Early in 1939 we made an attempt to check the hypothesis by comparing the relative magnitudes of action and resting potentials in a single nerve fibre. We expected to find that the resting potential would be slightly larger than the action potential, since it was known that the membrane resistance has a finite though small value during activity (Cole & Curtis, 1939). Instead of the expected result we found the action potential to be much larger than the resting potential. The first measurements were made with crustacean axons and external electrodes, but were not completely satisfactory because they did not give the absolute magnitudes of the potential difference across the nerve membrane. A method of inserting microelectrodes into squid axons was developed, and direct measurements of action and resting potentials were obtained in the summer of 1939 (Hodgkin & Huxley, 1939). At about the same time, Curtis & Cole (1940) evolved a rather different technique for performing the same experiment, and in a subsequent paper (Curtis & Cole, 1942) concluded that the action potential exceeds the resting potential by a large amount. The present paper provides an independent confirmation of this conclusion and extends it to crustacean nerve fibres. All the experiments to be described were made in 1939 and the bulk of the paper was written in 1940. Our measurements were less extensive than those of Curtis & Cole, but the results obtained seem sufficiently important to justify independent publication.

Apparatus and method

Preparation of fibre

The giant fibre in the first stellar nerve of *Loligo forbesi* was used. The best results were obtained with the fibres from large specimens (mantle length 10 in.), but usually the mantle was only 7 or 8 in. long. The diameter of the axons used varied between 450 and 550 μ.

The nerve trunk was tied near the ganglion and dissected out of the mantle as far as the first large branch of the giant fibre. Here the trunk was again tied and cut, and transferred to a dish



Text-fig. 1. Diagram of recording cell. *A*, fixed plate of mechanical stage of microscope. *B*, movable plate of mechanical stage. *C*, shelf attached to *B*. *D*, upper compartment of recording cell normally filled with sea water. *E*, lower compartment normally filled with oil. *F*, *F*, glass tubes conveying leads to stimulating electrodes. *G*, fibre. *H*, cannula. *I*, micro-electrode. *J*, *J*, glass plates forming partition between compartments. (The fibre passes through a hole formed by notches which have been ground in the edges of the glass plates.) *K*, handle for closing partition. *L*, holder for cannula. (An adjustable joint at the base of the holder is not shown.) The external electrode in the upper compartment and the arrangement of mirrors shown in Text-fig. 3 have been omitted.

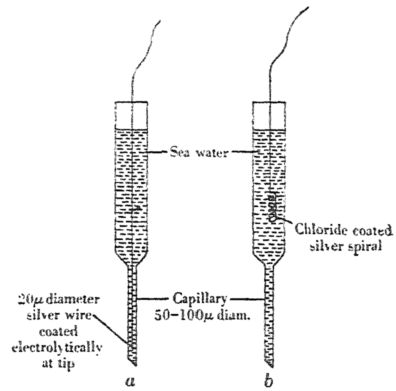
of sea water. In the first experiments the whole length of the fibre was cleaned, but later only a short length at one end was cleaned for the insertion of a cannula. In either case, when the dissection was finished, the axon was cut half through with a fine pair of scissors, and a cannula inserted and tied in with a silk thread. A fine loop of platinum wire was attached to the free end of the fibre. This acted as a weight and made the fibre hang vertically in the recording cell. The dissection and cannulation took about 2 hr., and were carried out under a binocular dissecting microscope.

Special apparatus

A solidly built microscope, with its optical axis horizontal, was clamped to the baseboard of a micromanipulator. The transverse movement of the mechanical stage was removed, and a horizontal shelf was fixed to the vertically moving plate of the stage. A recording cell, shown diagrammatically in Text-fig. 1, rested on this shelf.

The recording cell consisted of two compartments separated by a glass partition which could be opened or closed by a handle passing through the wall of the cell. The lower, smaller compartment contained the platinum-wire stimulating electrodes and was always filled with medicinal paraffin oil. The upper compartment could be filled with either sea water or paraffin; in the former case, the glass partition prevented the oil in the lower compartment from floating to the surface.

The microelectrode usually employed is shown in Text-fig. 2*a*. It consisted of a glass capillary about 4 cm. long. One-half of this had an external diameter slightly less than 0·1 mm. while the other was about 0·5 mm. in width. A 20μ. diameter silver wire was passed to within about 1·5 mm. of the tip, the capillary was filled with sea water and the wire coated electrolytically with silver chloride. This type of electrode had an electrical resistance of about 50,000 ohms. A different form of electrode is shown in Text-fig. 2*b*. This electrode gave a steadier potential, but it had an inconveniently high resistance (about 2 megohms) and was only used on one occasion. The microelectrode was suspended by a short length of wire from the tip of a glass rod held in one of the micromanipulator carriages.



Text-fig. 2. Types of microelectrode employed.

Sea water was normally used in the upper compartment, and in this case the external electrode consisted of a chloride coated coil of silver wire immersed in the sea water. When paraffin oil was used, an agar wick electrode was brought into contact with the outside of the fibre opposite the tip of the microelectrode.

Procedure

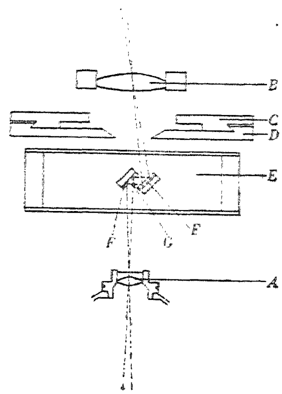
The fibre was transferred to the recording cell when the dissection and cannulation were complete. The cannula was attached to a clip which was mounted on the vertically moving plate of the microscope by means of a small universal joint. This was adjusted until the fibre hung vertically with its far end in the paraffin oil in the lower compartment of the recording cell. The partition was closed, and the upper compartment was filled with sea water. The stimulating electrodes were brought into contact with the fibre.

The vertical movement of the stage and the mount of the cannula were then adjusted so that the tip of the cannula was in the field of the microscope, which was fitted with a 2 in. objective. The microelectrode was lowered into the cannula by means of the micromanipulator till its tip was also visible. The mechanical stage was then racked up, raising the cell and cannula, and thus drawing the fibre up round the microelectrode, whose tip remained in the field of view. If one side of the fibre began to approach the microelectrode too closely, the cell was moved horizontally in that direction. Since the lower end of the fibre passed through the small gap left between the two pieces of the partition, this manœuvre carried that side of the fibre away from the microelectrode.

It was still impossible, however, to tell when the microelectrode was in danger of touching the front or back of the fibre, and at first about half the fibres were spoilt by this accident. A pair of small mirrors made of pieces of coverslip backed with air, were therefore mounted as in Text-fig. 3, so that a side view of the fibre was seen in the field of the microscope at the same

time as the front view. There is no particular virtue in the use of airbacked mirrors, and the only reason for employing them was that front-silvered mirrors were not available. The mirrors were attached to a glass rod held in the second micromanipulator carriage. With this technique it was possible to ensure that the microelectrode did not touch the surface of the nerve fibre at any point. Photographs showing the microelectrode in position are reproduced on Pl. 1.

The giant fibre was stimulated repetitively during the process of lowering a microelectrode into its interior. A small action potential was observed while the microelectrode was still inside the cannula and this gradually increased as the electrode was lowered, until it reached a constant amplitude at a distance of 10 mm. from the cut end.



Text-fig. 3. Diagram of optical arrangement, seen in plan. *A*, 2 in. objective in tube of microscope. *B*, low-power condenser in substage. *C*, fixed plate of mechanical stage. *D*, movable plate of mechanical stage. *E*, recording cell. *F*, *F*, mirrors. *G*, axon. The paths for the central rays for the two images of the fibre are shown as dotted lines.

Electrical recording system

Potentials were recorded by means of a cathode-ray oscillograph and a balanced direct-coupled amplifier. This was designed for us by Dr Rawdon Smith of the Psychological Laboratory, Cambridge, and proved completely satisfactory. Calibrations showed that the amplifier response was constant to within less than 5% between 0 and 10,000 cyc./sec. In order to keep the input impedance as high as possible, the recording leads were connected to the grids of two valves which were placed about 6 in. from the preparation. These valves were arranged as cathode followers and produced no amplification; they were used solely as an impedance matching device. Calibrations of the input stage and the amplifier were made with electrically generated rectangular pulses.* The wave form of these pulses was first tested by applying them directly to a cathode-ray oscillograph. This showed that the deflexion was 90% complete in 10 μsec. The pulses were then reduced in amplitude by a low-resistance attenuator and fed into the input of the amplifier. Under these conditions the deflexion was 90% complete in 20 μsec. Hence the amplifying system was sufficiently rapid to follow an action potential lasting 500–1000 μsec.

The d.c. resistance of the input stage was of the order of 10^{10} ohms and the grid current 10^{-10}–10^{-11} amp. The a.c. input impedance was mainly determined by the stray capacity of the grid lead which was less than 20 $\mu\mu$F. A stray capacity of this magnitude might have introduced a serious error, if the action potential had been recorded through a resistance of several megohms, but it was of no importance in our experiments, because the total resistance of preparation and electrodes was less than 100,000 ohms.

The linearity of the amplifier was checked by applying a voltage which could be varied between 0 and 100 mV. in steps of 1 mV. This test showed that the deflexion of the oscillograph was directly proportional to the input to within 5% over the range of voltages used experimentally.

The standard voltages used for calibrating the oscillograph were obtained by passing a current of 100 μa. through Muirhead decade resistances. The current was set to exactly 100 μa. with a microammeter which had previously been calibrated by means of a Weston standard cell and decade resistances. All the resistances used had been tested by the National Physical Laboratory and were correct to within 0·1%.

Brief thyratron discharges were used for stimulating. These were synchronized with the sweep circuit and applied to the nerve through a small transformer.

* The pulse-generating apparatus was built up by Dr Rushton, and these calibrations were made while one of us was collaborating with him in another research.

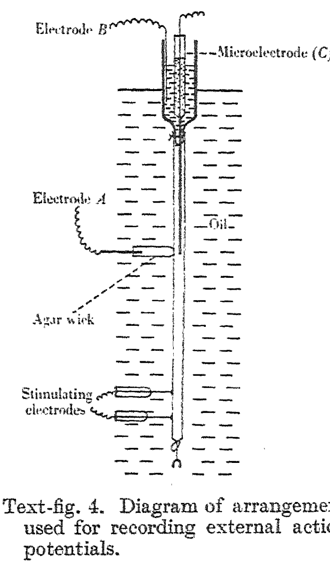
Experimental results

Preliminary experiments with internal electrodes

As soon as the method of inserting microelectrodes began to be successful, tests were made to see if the presence of an internal electrode caused any change in the activity of the fibre. An axon was set up in oil as shown in Text-fig. 4 and potentials were recorded between electrodes *A* and *B*. *A* was a conventional type of external electrode, while *B* behaved like an external electrode in contact with the cut end of a nerve fibre. The record obtained from these two electrodes was an ordinary monophasic action potential whose magnitude depended upon the amount of short-circuiting introduced by the external fluid. Action potentials were recorded before and after the introduction of the microelectrode, and the magnitudes of the two potentials compared. A typical result was as follows:

Action potential before insertion of microelectrode = 28·5 mV.
After insertion = 28·25 mV.

The difference between the two action potentials was within experimental error, and in some experiments the recorded potential was actually slightly greater after the insertion of the microelectrode. This test indicated that the microelectrode had little effect on the activity of the axon. Further confirmation of this point came from the fact that the action potential was conducted without decrement for a considerable distance beyond the tip of the microelectrode. In one experiment a normal action potential was recorded at a distance of 9 mm. above the tip of the electrode.



Text-fig. 4. Diagram of arrangement used for recording external action potentials.

Size of microelectrode

At an early stage in the investigation we made experiments to determine the optimum size for the microelectrode. Large electrodes are convenient, because they are easy to make and have a low electrical resistance; on the other hand, they are more likely to damage the axon than small ones. In order to find out how large an electrode could be tolerated, we prepared a series of glass rods with diameters of 30, 70, 100 and 170 μ. These were inserted into the inside of the axon and the external action potentials recorded in the usual way. The 170 μ. rod produced a 10% decrease in the size of the action potential, but the smaller rods had no effect on the magnitude of the potentials.

We concluded from this experiment that it was safe to use electrodes with diameters up to about 100μ. At first it seems remarkable that an electrode with a diameter equal to one-fifth of that of the axon should have no effect on the activity of the axon. However, the volume of axoplasm displaced by such an electrode would be very small, since the cross-sectional area of the fibre is twenty-five times greater than that of the electrode. When a micro-electrode is inserted, it must either displace axoplasm into the cannula or cause the axon to swell. The second alternative is probably correct, since there was no indication of a flow of axoplasm into the cannula. We did not notice any change in diameter, but this is not surprising since the increase would not have amounted to more than 2% of the axon diameter.

Comparison of action potential obtained with internal and external electrodes

With the arrangement of Text-fig. 4 it was easy to compare the magnitudes of the action potential recorded with internal and external electrodes. A typical result was as follows:

Axon in oil: Action potential recorded externally from electrodes A and $B = 28$ mV.

Action potential recorded across membrane from electrodes A and $C = 72$ mV.

In this experiment the axon was left with considerable amounts of material attached to it. Hence the potential recorded with external electrodes was greatly reduced by short-circuiting effects.

One great advantage in using an internal electrode is that the axon can be kept in sea water and need not be surrounded by an insulating material. With external electrodes no action potential can be recorded from an axon immersed in sea water; with an internal electrode the potential is recorded directly across the membrane and is independent of the external resistance. This is illustrated by a continuation of the experiment just described. The oil was replaced by sea water and the potential recorded again:

Axon in oil: Action potential recorded across membrane = 72 mV.

Axon in sea water: Action potential recorded across membrane = 74 mV.

Characteristics of the action potential

When the microelectrode had been inserted, it was a simple matter to obtain the absolute magnitude of the action potential. The fibre was stimulated at the lower end and the response measured directly on the oscillograph screen or recorded photographically. The amplitude of the action potential was then obtained by calibrating the oscillograph with a known input voltage. Errors which might have arisen from non-linearity of the amplifier were

avoided by increasing the calibrating voltage until it gave a deflexion equal to that produced by the action potential. The results obtained with ten axons are given in Table 1. Some of these fibres were in poor condition and therefore gave small action potentials. Those which produced potentials greater than 80 mV. appeared to be in perfect condition for they remained excitable and transmitted impulses for many hours.

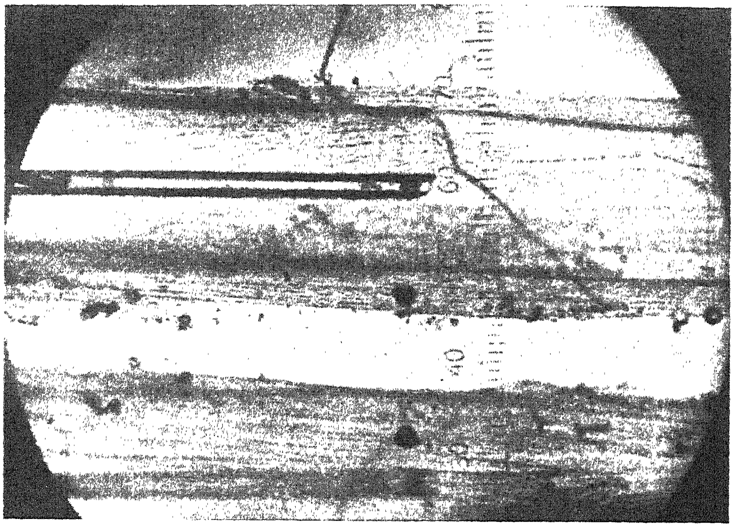
TABLE 1. Action and resting potentials recorded with a microelectrode. The potentials tabulated are those obtained at the beginning of each experiment

Axon	Action potential mV.	Resting potential mV.	Positive after potential mV.
1	57	—	—
2	95	47	15
3	79	42	13
4	71	30	—
5	58	—	—
6	65	—	—
7	80	—	—
8	68	35	—
9	86	47	15·7
10	92	46	15

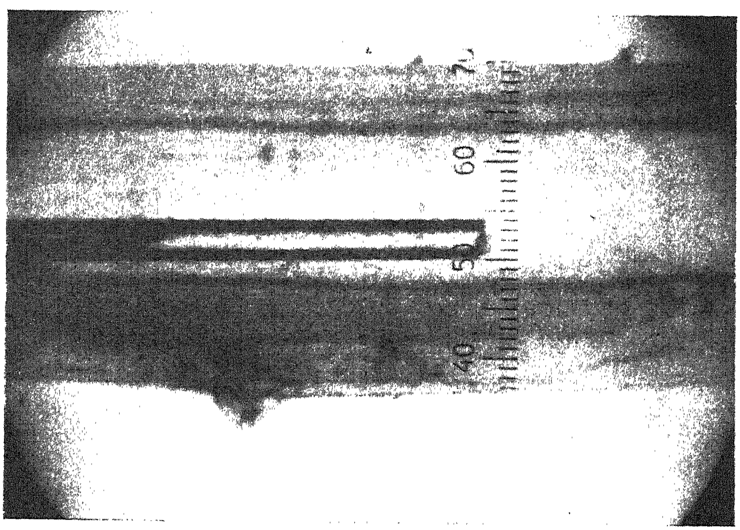
A typical record of an action potential, with an amplitude of 85 mV., is shown on Pl. 2. Here the negative phase of the action potential lasts for 0·7 msec. and is followed by a positive phase with an amplitude of 14 mV. and a duration of about 2·5 msec. This positive phase has been observed by everyone who has worked with squid axons (Cole & Curtis, 1939; Hodgkin, 1939; Pumphrey, Schmitt & Young, 1940). At first it was thought to be a diphasic artifact arising at the cut end of the axon. However, as Curtis & Cole (1940) have pointed out, the fact that it can be recorded with an internal electrode proves that it must be a genuine part of the response.

The resting potential

The measurement of the resting potential was complicated by the fact that the electrode potential of the microelectrode was not always the same as that of the external electrode. The potential difference (p.d.) between the two electrodes usually remained constant, but it could not be relied on and it was necessary to check the electrode p.d. at frequent intervals during the course of an experiment. The following procedure was therefore adopted. The microelectrode was placed in the sea water outside the axon and its potential measured against the external electrode. It was then removed from the sea water and lowered through the cannula into the axon. As the electrode was inserted, its potential became increasingly negative until it reached a constant value at a distance of about 6 mm. from the cannula. Measurements were usually made at a distance of 8–9 mm. from the cannula. As soon as values for the action and resting potentials had been obtained, the microelectrode was withdrawn, replaced in sea water and its potential remeasured.



(b)



(a)

Photomicrographs of electrodes inside giant axon. Adjacent tissues have not been completely removed from the giant fibre which shows as a clear space. One scale division equals 33μ. (a) Photomicrograph taken without double mirror device. (b) Photomicrograph taken with double mirror. The right-hand image is formed directly by the microscope, while the left-hand image is a side view seen in the small mirror.

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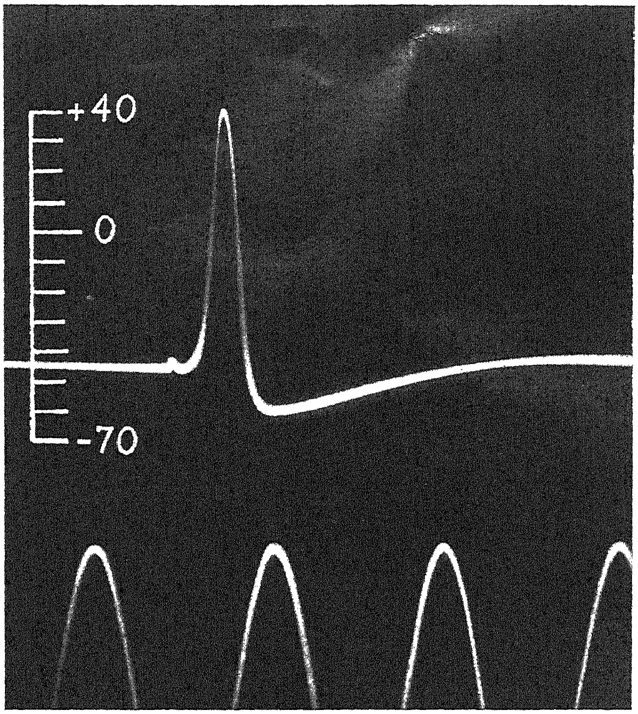


Action potentials recorded between inside and outside of axon. Time marker 500 cyc./sec. The vertical scale indicates the potential of the internal electrode in millivolts, the sea water outside being taken as at zero potential.

This was done in order to make certain that the electrode potentials had not altered during the course of the experiment. The resting potential was obtained from the difference between the potential of the microelectrode inside the fibre and its potential in the sea water. This method proved quite satisfactory, and values obtained in successive tests agreed reasonably well with one another. The following measurements were taken from an experiment in which the microelectrode was inserted and withdrawn on four successive occasions:

Resting potential (mV.)	Action potential (mV.)
44	83
41	81
41	81
44	83

Values for resting potentials obtained from six axons are given in Table 1. These results show that the resting potential was always much smaller than the action potential. The two sets of measurements are strictly comparable, since they were obtained at the same time and from the same point on the axon.

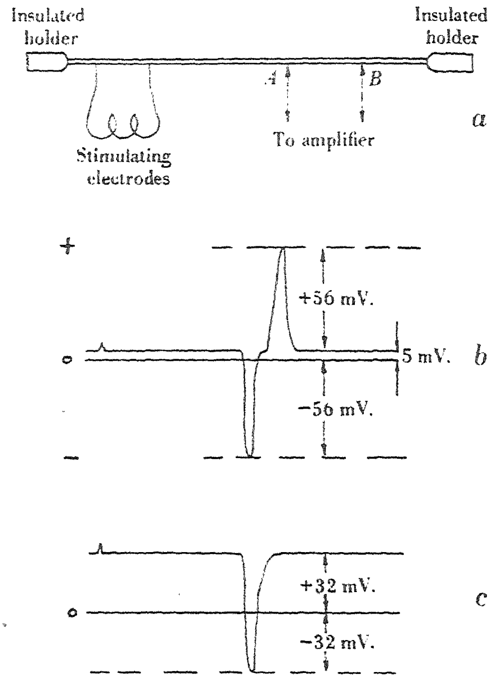
The difference between resting and action potentials is illustrated by Pl. 2. Here the resting potential was found to be 44 mV. Experimentally, this means that when the microelectrode was withdrawn and placed in the surrounding sea water, the oscillograph beam moved from the resting level to the point marked zero on the record. On the other hand, when the fibre was excited the deflexion overshot the resting level and the microelectrode then became 40 mV. positive to the surrounding sea water.

Experiments with external electrodes

Further evidence for the conclusion that the action potential overshoots the resting potential is provided by experiments with external electrodes. Using this technique, measurements were made on the nerve fibres of crabs and lobsters as well as of squids. A typical experiment with an axon from the lobster (*Homarus vulgaris*) is summarized below. A length of about 3 cm. of a $70\,\mu$. axon was dissected from the nerve trunk in the meropodite of one of the walking legs of a lobster. It was then set up in an electrode assembly and immersed in paraffin oil. The general characteristics of the electrode system were similar to those described in an earlier paper (Hodgkin, 1938) except that silver-silver chloride electrodes terminating in agar wicks were used in place of fine platinum wires. Stimulating and recording electrodes were arranged in the manner shown in Text-fig. 5*a*. At the beginning and end of the experiment a test was made to find if there was any residual p.d. between the electrodes. This was done by immersing both electrodes in sea water and recording the p.d. between them. The p.d. was found to be less than 0·4 mV. and could therefore be ignored. In order to observe action

potentials, the electrode assembly was raised into the layer of paraffin oil and the fibre stimulated repetitively. The record obtained is shown in Text-fig. 5*b*.

The resting potential at *A* was 5 mV. positive with respect to *B*, an asymmetry which can probably be attributed to the proximity of the cut end of the axon. When the action potential travelled along the nerve fibre, electrode *A* first became 56 mV. negative to *B*; later the potential wave reached *B* which



Text-fig. 5. Resting and action potentials in *Homarus* axon. Experiment of 16 July 1939. (*a*) Diagram of electrode assembly. The distance between electrodes *A* and *B* was about 10 mm. (*b*) Sketch of action potential recorded on C.R.O. (voltage at *A*) − (voltage at *B*) shown as positive. Normal nerve at *A* and *B* electrodes. (*c*) As in (*b*) but with isotonic potassium chloride placed on electrode *B*.

then became 61 mV. negative to *A*. A drop of isotonic potassium chloride solution was then placed on the nerve at *B*. This caused a transient discharge of nerve impulses and a steady resting p.d. of 32mV. between *A* and *B*. On stimulation, record *C* was obtained. The action potential at *B* had disappeared while that at *A* amounts to 64 mV. Hence the active nerve becomes 32 mV. negative to a part which has been depolarized with isotonic potassium chloride.

Figures from a similar experiment with a 30 μ. diameter axon from *Carcinus maenas* are given in Table 2.

TABLE 2. Experiment of 15 February 1939. Electrode assembly as in Text-fig. 5*a*. The distance between electrodes *A* and *B* was about 7 mm.

Condition of nerve: Electrode *A*	Condition of nerve: Electrode *B*	Potential difference (mV.) $A-B$
At rest	At rest	+3·2
Action potential	At rest	−68
At rest	Action potential	+65
At rest	Isotonic KCl applied	+37·5
Action potential	Isotonic KCl applied	−27·5

A typical result with a squid axon and external electrodes is seen in Table 3.

TABLE 3. Experiment of 8 August 1939. Electrode assembly as in Text-fig. 5*a*. The distance between electrodes *A* and *B* was about 15 mm.

Condition of nerve: Electrode *A*	Condition of nerve: Electrode *B*	Potential difference (mV.) $A-B$
At rest	At rest	+7
Action potential	At rest	−67
At rest	Action potential	+70
At rest	Isotonic KCl applied	+43·7
Action potential	Isotonic KCl applied	−23·3

Several experiments were made in order to discover whether the axon was fully depolarized by the action of isotonic potassium chloride. The method employed was to observe the depolarization produced by various processes which might damage or destroy the nerve membrane and to compare the results obtained with that produced by isotonic potassium chloride. The following methods were tested:

(1) Cutting half through a giant fibre.
(2) Cutting clean through a giant fibre or crustacean fibre.
(3) Crushing with fine forceps.
(4) Application of chloroform solution.

No significant difference was observed between the results obtained by these four processes. The depolarization observed was always less than that produced by isotonic potassium chloride. In crustacean axons the difference between the two e.m.f.'s was about 4 mV., in squid axons it was sometimes as great as 10 mV. The difference is explained by Curtis & Cole's (1942) observation that isotonic potassium chloride reverses the membrane potential. Curtis & Cole obtained reversed potentials of 15 mV., but a smaller value would be recorded with external electrodes since the membrane potential would be reduced by the short-circuiting effect of the external fluid.

The distribution of potential in the fibres which had been treated with potassium chloride was rather complicated. The potassium chloride solution was applied to the outside of the nerve fibre, but did not replace the sea water in the wick electrodes. There was therefore a junction potential of approximately 4·5 mV. between the outside of the nerve fibre and the sea water in the electrode *B* (Text-fig. 5): the sign of this p.d. being such that the electrode was positive to the nerve. This was partly compensated by the junction potential between the potassium chloride

at B and the sea water round the normal nerve at A. The contribution of this e.m.f. to the total recorded potential must have been less than 4·5 mV., because of the short-circuiting effect of the interior of the nerve fibre. The reduction in potential can be assessed by applying the standard equations of cable theory, which indicate that the potential would be reduced in the ratio r_2/r_1+r_2, where r_1 and r_2 are the resistances per unit length of the external fluid and the interior of the nerve fibre. Finally, there was the reversed p.d. across the membrane in the potassium chloride region. This may be taken as 15 mV., but its effect on the total recorded potential must have been reduced by the short-circuiting action of the external fluid in the ratio r_1/r_1+r_2. The effect of these three different sources of e.m.f. can be assessed if an assumption is made as to the ratio of r_1 to r_2. If $r_1=r_2$ the net p.d. amounts to 5·25 mV. If $r_1 \gg r_2$ it amounts to 10·5 mV. In both cases the sign of the p.d. is similar to that observed experimentally.

The results of the experiments with external electrodes are summarized in Table 4. They indicate that the action potential is roughly 75% greater than the injury potential produced by isotonic potassium chloride solution. The difference between the action potential and the true resting potential must have been greater than this, since isotonic potassium chloride itself reverses the membrane potential.

TABLE 4. Results obtained with external electrodes. The injury potentials were produced by applying isotonic potassium chloride to the nerve fibre. The figures in the bracketed rows were obtained from different parts of the same nerve fibre. All the observations were made from records similar to that shown in Text-fig. 5*c*

Date of experiment	Type of axon used	Action potential mV.	Injury potential mV.
{3. ii. 39	Axon from *Carcinus maenas*, *ca.* 30 μ. in diameter	62	36
{3. ii. 39	" "	62	42
3. ii. 39	" "	54	36
5. ii. 39	" "	55	39
9. ii. 39	" "	62	34
9. ii. 39	" "	73	37
10. ii. 39	" "	55	39
14. ii. 39	" "	49	28
15. ii. 39	" "	65	37·5
24. v. 39	Axon from *Homarus vulgaris*, *ca.* 70 μ. in diameter	52·5	36
16. vii. 39	" "	64	32
4. viii. 39	Axon from *Loligo forbesi*, *ca.* 500 μ. in diameter	78	49·5
8. viii. 39	" "	67	43·7
11. viii. 39	" "	54	36

Comparison with the work of Curtis & Cole (1942)

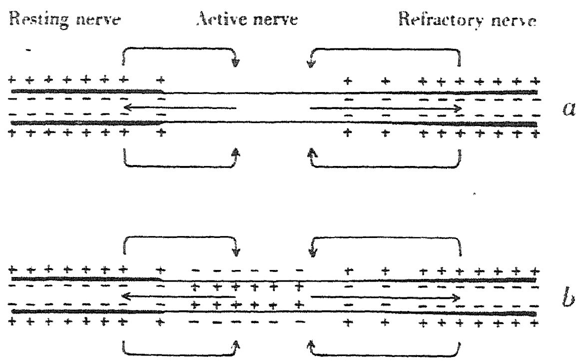
The experiments described in this paper are in general agreement with those of Curtis & Cole (1942), in that they show that the action potential overshoots the resting potential. But there is considerable difference between the numerical results obtained in the two sets of experiments with internal electrodes. The largest action potential recorded by us was 95 mV., whereas Curtis & Cole report action potentials as high as 168 mV. and give an average value of 105 mV. No certain explanation of the difference between the two sets of results can be offered. Curtis & Cole's experiments were probably carried out at a higher temperature, but this could not account for more than a fraction of the difference obtained. We are inclined to believe that the

axons studied by Curtis & Cole were in better condition than ours. The preparation of giant axons is a very difficult operation, and the percentage of nerve fibres in perfect condition is likely to be rather small. The action potential is known to be sensitive to fibre condition. and it is possible that none of the action potentials studied by us were really of normal size. Curtis & Cole made much more extensive studies and their results may have approached more closely to the normal.

The resting potentials obtained are in better agreement. Curtis & Cole's average value is 51 mV., whereas ours is 41 mV. Some of this difference must be attributed to the type of electrode employed. Curtis & Cole used isotonic potassium chloride in the microelectrode whereas we used sea water. Hence Curtis & Cole's results should have been higher by the junction potential (sea water-potassium chloride) which is approximately 4·5 mV.: the remaining difference of 5·5 mV. is not statistically significant.

Discussion

The evidence described in this paper indicates that the membrane potential is reversed and that the inside of the nerve becomes positive during the passage of an impulse. The classical picture of nervous action must therefore be altered from the familiar form illustrated in Text-fig. 6*a* to that shown in Text-fig. 6*b*.



Text-fig. 6. Diagram illustrating (*a*) classical and (*b*) revised concepts of nervous activity.

Before accepting such a drastic revision of classical theory we shall do well to examine a more conventional explanation of the experimental results. The ionic composition of the interior of the nerve fibre is known to be very different from that of sea water (Webb & Young, 1940; Bear & Schmitt, 1939), so there should be a substantial junction potential between microelectrode and axoplasm. It can be argued that this junction potential might be of sufficient magnitude to reduce a membrane potential of 100 mV. to one of 50 mV., and in this way there seems at first some hope that the classical

picture of nervous activity can be retained. This argument fails for two reasons. In the first place it is very difficult to imagine how a junction potential of 50 mV. could be produced at the sea-water axoplasm interface, and still harder to account for the 100 mV. necessary to explain some of Curtis & Cole's (1942) results. In the second place we can show that such a junction potential would probably not influence the observed results even if it were of the right order of magnitude. According to the conventional membrane theory the nerve surface becomes freely permeable to all ions during activity and can so contribute nothing to the recorded p.d. The active nerve may therefore be considered as a symmetrical cell in which sea water makes contact with axoplasm both at the boundary of the microelectrode and at the surface of the nerve. The junction potentials at these two surfaces must therefore cancel one another, and no p.d. should be recorded between the inside and outside of the axon.

A qualitative explanation of the reversal of potential can be obtained if it is assumed that the surface membrane changes from a condition of rest in which it is permeable only to K^+ to one of activity in which it is freely permeable to K^+ and Na^+ but not to Cl^-. In this case there would be a diffusion potential across the surface membrane and an uncompensated junction potential between axoplasm and sea water at the boundary of the microelectrode. If the former potential were smaller than the latter, or of opposite sign, the p.d. recorded during activity would be reversed. The extreme magnitude of this effect can be assessed in the following way. Consider first the junction potential between sea water and axoplasm. The approximate concentrations of K^+, Na^+ and Cl^- are known (Webb & Young, 1940; Bear & Schmitt, 1939), but there is a deficit of anions which is usually taken to indicate the presence of a large fraction of organic anions in the cell. For the purposes of calculation Curtis & Cole (1942) assume that these anions are monovalent and that their mobilities are such as to make the conductivity of axoplasm equal to its measured value, the mobilities of the remaining ions being taken as equal to those in sea water and their concentrations as equal to those reported by Bear & Schmitt (1939). With these assumptions they conclude that the junction potential $E_{\text{isotonic KCl}} - E_{\text{axoplasm}}$ would be $+6$ mV. This indicates that the junction potential $E_{\text{sea water}} - E_{\text{axoplasm}}$ would be about $+10{\cdot}5$ mV. We made a similar calculation, but assumed that the missing anions had zero mobility and that the concentrations of the remaining ions were equal to those reported by Webb & Young (1940). Ionic mobilities were taken as equal to those in sea water, and Henderson's equation was used for computation (Henderson, 1907, 1908). These assumptions gave a value of $+19$ mV. for the junction potential $E_{\text{sea water}} - E_{\text{axoplasm}}$. A minimum value for the residual p.d. across the hypothetical active membrane can be calculated if it is assumed that the selective permeability of the membrane to

Na^+ and K^+ is entirely lost, and that the mobilities of these ions in the membrane are in the same ratio as those in water. With these assumptions we find that the active membrane potential would be about 7 mV., if the concentrations of K^+ and Na^+ inside the axon are as reported by Webb & Young (1940). The sign of this potential is such that the inside of the axon would be negative to the outside. We can now assess the total p.d. which would be established between external and internal electrodes. If we take a figure based on Curtis & Cole's (1942) calculation for the junction potential we find that the residual p.d. would be $7-10{\cdot}5=-3{\cdot}5$ mV. If our figure is taken it would be $7-19=-12$ mV. In both cases the sign of this p.d. would be such as to produce a reversal of e.m.f. during activity, but its magnitude would be quite insufficient to explain the 45 mV. recorded by us or the 100 mV. difference reported by Curtis & Cole. We must therefore conclude that the difference between action and resting potentials indicates a real reversal of potential at the surface membrane as indicated in Text-fig. 6*b*.

Four types of explanation can be advanced to account for the reversal of membrane potential during activity. These may be classified in the following way:

(1) The active membrane becomes selectively permeable to anions which are present in the axoplasm, but are in low or zero concentration in sea water.

(2) Activity involves a change in the orientation of dipoles in the surface membrane.

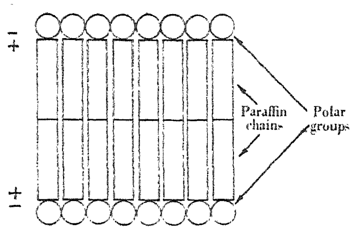
(3) Explanation in terms of apparent membrane inductance.

(4) Series capacity hypothesis.

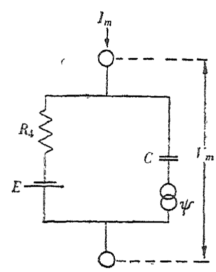
The first hypothesis is self-explanatory. As an example, let us assume that the axoplasm contains a large quantity of lactate in ionic form and that the membrane becomes freely permeable to these ions during activity. An alternative assumption is that the membrane does not change at all during the passage of an impulse and that activity consists in the liberation of an anion with high mobility in the membrane. In either case, anions would tend to diffuse out of the axon, but would be restrained at the surface membrane; hence a membrane potential of reversed sign would be set up. Such a state of affairs is theoretically possible, but does not seem at all probable, since it is hard to imagine that the concentration or mobility of lactate or any other organic ion would be sufficient to swamp the contributions of K^+ and Cl^- to the membrane potential.

The second hypothesis is based upon the fact that monomolecular films of fatty acids may change the p.d. of the air-water interface by several hundred millivolts. The direction of the p.d. indicates that the polar end of a fatty acid is negative with respect to the paraffin chain. The cell surface could not consist of a monomolecular film of fatty acid, since a single layer of fatty acid molecules would be unstable in contact with two aqueous phases. But

it might be composed of a bimolecular film of fatty acid or lipoid molecules orientated in the manner shown in Text-fig. 7 (see Danielli, 1936, and others). Protein molecules are almost certainly present as well as lipids, but have been omitted for the sake of simplicity. The orientated dipoles in a system of this kind would give rise to potential gradients at each side of the membrane. The two gradients would be of opposite sign and could therefore contribute little to the resting membrane potential. Even if the gradients were dissimilar they could make no permanent contribution to the resting potential, since ions are free to move through the surface, and would neutralize a p.d. arising from a purely static orientation of dipoles. But a transient wave of negativity would occur if the inner layer of dipoles were removed, or deorientated in some way when the membrane was excited. The duration of this wave would depend upon the rate at which the membrane capacity was discharged by



Text-fig. 7.



Text-fig. 8.

Text-fig. 7. Diagram showing hypothetical membrane composed of bimolecular layers of fatty acid. Positive and negative signs indicate the main potential gradients associated with each layer of molecules.

Text-fig. 8. Hypothetical equivalent circuit of nerve membrane.

ions, and the time constant of this process should be the same as the electrical time constant of the whole membrane. The time constant of the resting membrane in the squid axon is about 500 μsec., while that of an active one is about 25 μsec. (see Cole & Curtis, 1939; Cole & Hodgkin, 1939; Cole, 1941). The time scale of the process is therefore of the right order of magnitude to explain the overshoot of the action potential provided that the membrane e.m.f. is sufficiently large and develops sufficiently rapidly. The p.d. arising from a fatty acid such as butyric acid has a maximum value of about 350 mV., while values in excess of 600 mV. have been recorded from such compounds as triethylamine (Rideal, 1930). It therefore seems reasonable to suppose that an action potential of 100 mV. might arise in this way. An exact formulation of this theory is impossible, but it may be examined from a slightly more quantitative point of view. Let us assume that the action potential arises solely from changes in molecular orientation and that the nerve membrane has the equivalent circuit shown in Text-fig. 8. The resting membrane

e.m.f. is represented by the battery E, the voltage due to orientated dipoles by a variable ψ, the total p.d. across the membrane by V_m, and the current through it by I_m; R_4 and C are the membrane resistance and capacity respectively. These quantities are related in the following way:

$$I_m = \frac{V_m - E}{R_4} + \frac{Cd\,(V_m - \psi)}{dt}, \tag{1}$$

where I_m is the membrane current density in amp. cm.$^{-2}$,

C is the membrane capacity in F. cm.$^{-2}$,

R_4 is the membrane resistance in ohms cm.^{2},

E and V_m are expressed in volts and t in seconds.

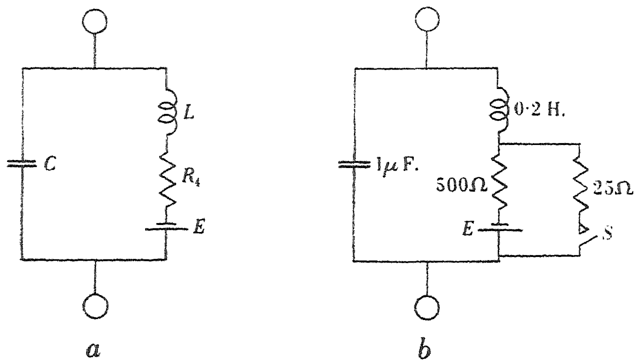
An approximate estimate of the value of $d\psi/dt$ at the crest of the action potential wave can be made in the following way. By definition dV_m/dt is zero and $V_m - E$ is equal to the peak amplitude of the action potential wave which may be taken as -100 mV. According to Cole & Curtis (1939) R_4 is about 25 ohms cm.^{2} at the height of activity while C remains constant at about 1 μF. cm.$^{-2}$. I_m can be obtained from the second derivative of the action potential wave to which it is related by equation (2) when the fibre is immersed in a large volume of sea water:

$$I_m = \frac{\rho}{2R_2}\,\frac{d^2V_m}{dx^2}. \tag{2}$$

In this expression x is distance along the fibre in cm., ρ is the radius of the fibre in cm., and R_2 the specific resistance of the axoplasm in ohms cm. Measurement of the peak of an action potential from an axon of radius 250 μ. indicated that d^2V_m/dx^2 was about 0·5 V. cm.$^{-2}$ R_2 was taken as 30 ohms cm., this being approximately the value found by Cole & Hodgkin (1938). Hence $I_m \doteqdot 0{\cdot}2 \times 10^{-3}$ amp. cm.$^{-2}$. When these values are substituted in equation (1), $d\psi/dt$ is found to be $-4{\cdot}2 \times 10^3$ V. sec.$^{-1}$. This result indicates that the rate of change of molecular orientation would be equivalent to that produced by a dipole layer with a potential difference of 420 mV. collapsing during a period of 0·1 msec. This is not an impossible assumption, although it is a little hard to imagine that such a change would leave the membrane capacity unaltered during activity.

A different type of explanation was put forward by Curtis & Cole (1942) to account for the difference between action and resting potentials. Their explanation is based upon the fact that the axial impedance of a squid axon appears to contain an inductive component (Cole & Baker, 1941). The inductance seems to be localized in the surface membrane and has a value of approximately 0·2 H. The quantitative results obtained were consistent with the assumption that the equivalent circuit of the membrane is similar to that shown in Text-fig. 9a (Cole, 1941). C is the fixed capacity of 1 μF. cm.$^{-2}$, while R_4 is a variable resistance which represents the rectifier element in the

nerve surface. Cole (1941) has shown that the equivalent circuit of Text-fig. 9*a* provides a possible explanation of the subthreshold response of a nerve fibre to rectangular pulses of direct current. When an anodic polarizing current or a weak cathodic one is applied, R_4 remains high enough to damp out the effect of L. But at larger cathodic currents R_4 ceases to mask L and the response shows a hump or local action potential which increases and tends to become oscillatory as the threshold is approached. The frequency of the oscillations is of the order of 400 cyc./sec. Cole suggests that the inductance may arise from a piezo-electric effect in the membrane; but as he points out, no ordinary piezo-electric system could have a natural frequency as low as 400 cyc./sec. We are reluctant to accept the idea of a genuine inductance in the membrane, since it is difficult to attach any physical significance to such

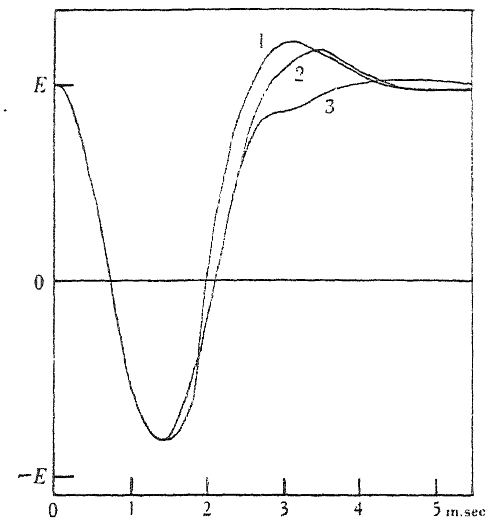


Text-fig. 9.

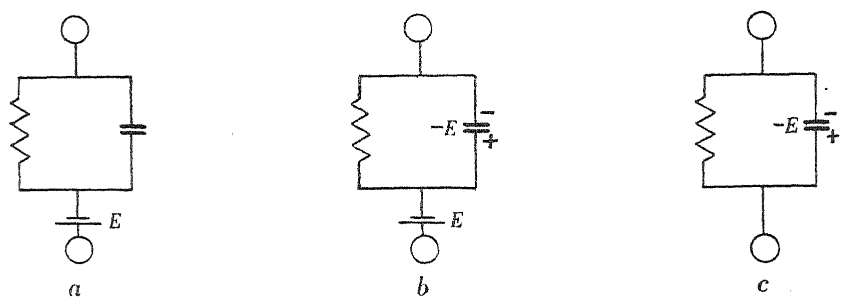
a concept. But an inductive element certainly offers an attractive explanation of the difference between action and resting membrane potentials. In order to illustrate how inductance might cause the action potential to overshoot the resting potential we assumed that the membrane had the structure of Text-fig. 9*b* and that excitation consisted in the closing of the switch S for a certain length of time. The potential waves resulting from three different periods of switching have been calculated and are given in Text-fig. 10. In the three cases considered, the reversed p.d. across the network is 80% of the battery voltage E. The action potential of nerve is much more complicated in its origin, but the simple model of Text-fig. 9*b* illustrates how inductance could cause the action potential to overshoot the resting potential. It also shows how an inductive element could explain the positive after potential.

The last explanation to be discussed will be called the series capacity hypothesis. It assumes that the resting membrane e.m.f. is in series with the membrane capacity instead of being in parallel with it as is normally supposed. The resting membrane is considered to have the structure shown in Text-fig. 11*a*. In the vicinity of an active region the p.d. across the membrane is

reduced, and for the purpose of this argument we shall assume that it is reduced to zero before excitation occurs. The membrane capacity will therefore acquire a reversed voltage $-E$ (Text-fig. 11*b*), and this will persist when the excitation occurs and the battery E disappears (Text-fig. 11*c*). A reversed



Text-fig. 10. Response of network shown in Text-fig. 9*b* to closure of switch. Curves show voltage (V) across network when switch is closed for following times. Curve 1: from $t=0$ to 1·4 msec. Curve 2: from $t=0$ to 2·13 msec. Curve 3: from $t=0$ to 2·8 msec. Initial boundary conditions taken as $V=E$ and $dV/dt=0$ when $t=0$.



Text-fig. 11.

action potential could arise in this way and should be capable of reducing adjacent parts of the nerve membrane to zero p.d. as was assumed initially. This hypothesis has not been developed in any detail and may not bear quantitative investigation. But it has one point in its favour. The membrane capacity is thought to correspond to a dielectric at least 20 Å. thick, whereas the voltage gradient may occupy only a few Å. The gradient is in any case

likely to be greatest at the edges of the dielectric and may conceivably have a low value over a large part of it. The idea of an e.m.f. in series with a capacity is therefore reasonable from a physical point of view.

Summary

1. A technique is described for introducing microelectrodes into the interior of giant axons from the squid (*Loligo forbesi*).

2. Action potentials recorded externally were not affected by the introduction of a microelectrode 100 μ. in diameter.

3. The action potential recorded across the surface membrane was not altered by changing the fluid surrounding the fibre from oil to sea water.

4. The amplitude of the action potential was found to be about twice that of the resting membrane potential. The largest action potentials recorded were about 90 mV., whereas the corresponding resting potentials were about 45 mV. All measurements were made with a microelectrode containing sea water at its tip, so that there must have been a junction potential between the axoplasm and the microelectrode. The true resting potential across the surface membrane was probably about 60 mV.

5. Measurements with external electrodes were made on single nerve fibres from crabs (*Carcinus maenas*) and lobsters (*Homarus vulgaris*) as well as on squid axons. These results showed that the action potential was about 75% greater than the injury potential produced by isotonic potassium chloride. The difference between the action potential and the true resting potential must have been greater than this, since isotonic potassium chloride itself reverses the membrane potential.

6. The difference between action and resting potentials is too large to be explained by a liquid junction potential between the axoplasm and the microelectrode. It must be due to a genuine reversal of potential at the surface membrane of an active section of nerve. Possible ways in which this reversal of potential might arise are discussed.

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THE EXCRETION OF UREA, SALTS AND WATER DURING PERIODS OF HYDROPAENIA IN MAN

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It is now well known that, even during periods of severe dehydration, urine continues to be formed. This is the so-called 'urine obligatoire' of Ambard & Papin (1909). This urine is concentrated and of high specific gravity and, if it is passed by a healthy person, usually freezes at about $-2{\cdot}2$ to $-2{\cdot}6^\circ$ C. There are, however, many unsolved problems about such urine, and statements have been made about it which are very difficult to reconcile with each other. In the first place, it is still not clear what regulates the volume of water passed by a dehydrated person. Ambard & Papin (1909) believed that it was the presence in the urine of one of the constituents at its limiting concentration, and that maximum concentrations of urea and salt could co-exist. 'Il exist une independance absolue des concentrations de l'urée et de NaCl.' Davies, Haldane & Peskett (1922) also considered that 'the simultaneous excretion of urea' by man was 'without effect on the kidneys' capacity for concentrating chlorides', and Gilman & Kidd (1938) that 'the presence of high concentrations of urea in the urine' of the dog 'had absolutely no effect on the osmotic ceiling of NaCl'. These statements imply that the kidney should be able to produce maximum concentrations of urea and salt simultaneously. If this were the case, it ought to be possible to demonstrate it during dehydration. Adolph (1923), however, did not do so consistently to his own satisfaction, and Chaussin (1920) found that the concentrations of urea and NaCl tended to vary inversely in concentrated human urine. McCance & Young (1944) confirmed this, and also showed that the volume passed during dehydration depended upon the intake of NaCl. They therefore suggested that, under the conditions of their experiments, the volume passed depended upon the total osmotic pressure of the urine rather than upon the concentration of any one constituent (Smith, 1937). Reference should perhaps be made here to the oft-quoted work of Gamble, McKhann, Butler & Tuthill (1934). These authors came to the conclusion that animals required less water to excrete urea than they did to excrete NaCl or other substances. This was

held to be an important feature of the water economy of terrestrial animals, and it was explained by 'the physical properties which urea exhibits to degrees which are almost unique'. In these experiments, however—and this has generally been overlooked—the rats were allowed to drink as much as they wished. The findings, therefore, would seem to have more bearing upon the mechanism of thirst production than of water excretion, and they certainly have no place in the present discussion. Secondly, Chesley (1938) found that, when urine volumes were below 0·35–0·5 c.c. per min., the percentage of urea was constantly at its maximum value for that person, and the clearances depended only upon the minute volume. He also found at these low volumes that (*a*) the creatinine, inorganic P and also the total solids were always maximally concentrated, (*b*) the clearances of endogenous creatinine, which some have considered to measure the glomerular filtration rates, varied directly and quantitatively with the urine volumes. McCance & Young (1944) did not find any evidence for a fixed percentage of urea when they manipulated the volumes by varying the intake of NaCl, for they always observed that the percentage of urea rose as the minute volume fell. Black, McCance & Young (1942), moreover, found that the glomerular filtration rates remained very stable even under conditions of considerable dehydration, and this again is difficult to reconcile with Chesley's results. It was accordingly decided to reinvestigate the secretion of urine during periods of water deprivation, and the results appear to be helpful and clear, although naturally all the conflicting statements cannot be reconciled.

Subjects and methods

The work was carried out between October 1943 and July 1944 on two healthy men, N.J., aged 30 and R.H., aged 20, and on one healthy woman, P.C., aged 28. The general plan was to bring the subjects into a state of mild dehydration in which the kidney was likely to be conserving water to its maximum capacity. Considerable variations in urine flow were then produced by a variety of methods and a study made of the changes in the plasma and urine.

After their usual supper the subjects drank nothing for the rest of the evening and, at a fixed time on the following morning, took a standard breakfast without anything to drink and came to the laboratory at 1 p.m. for lunch. This consisted of bread, butter and jam, but no liquids. On the experimental days they took one dose of urea, or of NaCl or of KCl at 10 a.m. and another larger dose at 12 noon. These substances were dissolved in as little water as possible and the solutions were almost saturated. On the control days nothing was taken between breakfast and lunch. On all days the urine formed between about 2.30 and 5 p.m. was collected as one specimen and blood was taken for analysis about 3.45 p.m. The experiments were carried out in this way to avoid the first diuresis and fall in urinary osmotic pressure which seem to be the immediate results of taking urea or salts (McCance & Young, 1944). After the urine had been collected at 5 p.m., the subjects had a meal, and with it as much to drink as they wished. Several days were usually allowed to pass before the next experiment. On two occasions the subjects were given salt-free diets for 3 or 4 days before the desired experiments were carried out. For special tests, water, and creatinine, were given by mouth, and small quantities of sucrose were injected intravenously. On one occasion 330 c.c. of 3% NaCl were administered intravenously in addition to 24 g. of NaCl by mouth.

On the samples of urine the following determinations were carried out. (1) The volume—in a cylinder of appropriate size. (2) The freezing-point—using a Beckmann thermometer. If precipitates formed, these were at first removed and redissolved, and the freezing-point of this solution was then separately determined and added to that of the original urine. This procedure was later discontinued as the correction was found to be a very small one. (3) The specific gravity—by a specific gravity bottle. This estimation was only carried out on about half the specimens. (4) Na, K and Cl by the methods given by McCance (1937) and McCance & Widdowson (1937). (5) Urea by Lee & Widdowson's (1937) method.

The blood was collected under paraffin, with precautions against venous stasis, and the plasma or serum separated without delay. On the serum the following estimations were made. (1) Na, Cl, urea and the freezing-point by the methods given above. The changes in the last were so small that this procedure was dropped after some 6 months. (2) For K about 2·5 c.c. serum were dried, then heated, without any additions, in a small crucible at 400–450° C. till the ash was perfectly white. 2·5 c.c. of N/10 HCl were then added and the ash dissolved at room temperature. Two samples, each of 1 c.c., were then taken and the K determined by Hubbard's (1933) method.

Results

Control data. On the standardized regimen which has just been described, it was impossible to detect any variations in the concentrations of Na, K, and Cl in the serum from one control day to the next, and the variations in urea were trifling. There were, however, quite definite changes in the urine, some of which are given in Table 1. It will be seen that the freezing-point

Table 1. Variations in the control urines from day to day

Subject	Freezing-point −° C.	Min. vol. c.c.	$\frac{\text{F.P.} \times 1000}{1\cdot86}$	Urine urea m.mol./l.	Urine Na m.equiv./l.	Urine Cl m.equiv./l.	Urine K m.equiv./l.	Urea + Na + Cl + 2 × K m.mol./l.
R.H. 1	2·42	0·52	1300	525	265	254	90	1225
2	2·60	0·63	1400	542	270	270	89	1260
3	2·67	0·46	1430	625	156	195	119	1214
P.C. 1	2·12	0·39	1140	433	170	173	82	940
2	2·35	0·32	1260	527	178	165	136	1142
N.J. 1	1·86	0·64	1000	394	200	192	57	900
2	2·14	0·46	1150	492	193	180	69	943
3	2·33	0·36	1260	542	137	171	94	1038
4	1·94	0·49	1040	421	165	195	50	881

of N.J.'s urine varied from −1·86 to −2·33° C., and of R.H.'s from −2·42 to −2·67° C. These variations in the freezing-point showed that the maximum osmotic work of which the kidney was capable was not nearly such a constant quantity as the concentrations of some of the constituents of the internal environment of the body. Adolph (1923) drew attention to the inconstancy of the urinary maxima for Cl and urea, and for the two together, and also pointed out that in spite of it Ambard had had the vision to establish the fact that there were these limiting concentrations.

There were variations of considerable size in the concentrations of some of the principal osmotically active constituents in these control urines. Thus, the urea in R.H.'s urine varied from 525 to 625 m.mol./l., and the K in that of N.J. from 50 to 94 m. equivalents/l. Sometimes the urea and salt varied inversely from day to day, but sometimes the changes were in the same direction and accompanied by a change in the freezing-point, and it was

clear that factors which were not being taken into account were producing these changes in the excretion of electrolytes and urea.

When the freezing-points were multiplied by the conventional 1000/1·86, and the results compared with those obtained by adding the urea, Na, Cl and $2 \times K$, it was evident that there was a rough relationship between them. The former exceeded the latter by 75–216 m.mol./l. Urea, Na and K salts, therefore, accounted for most of the osmotic activity of these urines, while undetermined substances were responsible for the remaining 10–20 %.

Serum urea. McCance & Young (1944) observed a considerable fall in the excretion of Na and of Cl when 35 g. of urea were injected intravenously into a dehydrated woman. No change was detected in the serum Na but there was a large fall in the serum Cl. Adolph (1925) probably had a similar phenomenon under observation. 50 g. of urea by mouth did not produce such an effect in these three subjects, but some evidence of a reciprocal change was noted in the serum chemistry of two of them. It was best shown by R.H., and data from his experiments are given in Table 2. It will be seen that the

TABLE 2. The effect of NaCl and of $NaHCO_3$ on the serum urea of a man (R.H.) who was not fully hydrated

Nature of experiment	Serum urea mg./100 c.c.
Control	42·2
15 g. NaCl by mouth	34·5
Control	42·0
25 g. NaCl by mouth	32·8
21 g. $NaHCO_3$ by mouth	37·3
Control	42·5
21 g. $NaHCO_3$ by mouth	36·0
30 g. NaCl by mouth	32·7

The experiments are given in the order in which they were performed.

serum urea was always lower on the days on which the doses of NaCl had been administered, and that $NaHCO_3$ appeared to have a similar effect. It has been suggested that these salts were so nauseating that they delayed the passage of lunch from the stomach and hence the formation of urea from the protein in that meal. This is not a very satisfactory theory because the small doses of K salts were quite as nauseating but they did not depress the serum urea. If, however, this simple explanation is not accepted it is as difficult to explain these observations in terms of conventional body chemistry and membrane permeability as it is to explain those of McCance & Young (1944) or of Adolph (1923). Nevertheless, it may be helpful to place them on record.

The excretion of urea. The composition of the control urines has been given in Table 1. The effect of a 'salt-free' diet for a few days was to reduce to a very small figure the Na and Cl in the urine. The accompanying changes were a fall in the minute volume of the urine—see Table 3, a rise in the percentage of urea in the urine, and a rise in the urine/plasma urea ratio. There was no

TABLE 3. The relationship between the excretion of urea and that of other osmotically active substances

Subject P.C.

Nature of diet and experiment	Blood urea mg./100 c.c.	Blood urea m.mol./l.	Urine Min. vol. c.c.	Urine Urea m.mol./l.	Urine Na ×2 m.mol./l.	Urine K ×2 m.mol./l.	Urine Sum of urea + 2 (Na + K)	Urine $\frac{\text{F.P.} \times 1000}{1{\cdot}8}$
Controls (average)	25·3	4·22	0·355	480	346	224	1050	1200
,, +25 g. of urea	61·8	10·30	0·830	835	133	91	1059	1200
,, +50 g. of urea	112·0	18·60	1·72	752	150	67	969	1050
Low salt (average)	23·0	3·94	0·24	662	49	212	923	1200
,, +25 g. of urea	66·2	11·00	0·80	860	9	149	1018	1090
Control + 25 g. urea and 15 g. NaCl	65·0	10·80	2·20	409	452	95	956	1100

change in the averaged freezing-points. These results, which were anticipated from the findings of McCance & Young (1944), may be explained as follows. The salt-free diet reduced the number of osmotically active substances to be excreted per minute and, since the kidney continued to concentrate the urine to the same extent as before, there was also a fall in the volume of water excreted per minute. The percentage of urea in this water naturally rose.

Urea was taken by mouth both when the subjects were on normal and on salt-free diets, and P.C.'s data are given in Table 3. The findings were (1) a rise in the concentrations of urea both in the blood and urine, (2) a rise in the minute volume of the urine, (3) a fall in the percentage of Na and K salts in the urine, (4) no consistent change in freezing-point over a range of volumes up to 1·72 c.c./min. If the small fall at that volume is significant, it may be attributed to the hurried passage of the urine through the distal tubules (McCance & Young, 1944). It is suggested that these changes were brought about as follows. The rise in the blood urea caused a considerable increase of urea molecules to appear in the distal tubules per minute. The kidney continued to concentrate the urine to the limit of its capacity, but the osmotic activity of the urea forced the kidney to excrete much more water per minute and the concentration of Na and K salts in this water naturally fell.

The evidence then is that, in mild dehydration, the volume of the urine passed per minute depends upon the number of osmotically active substances claiming excretion per minute (*a*) at the lowest minute volumes when the urine is free of salt, (*b*) when the urine contains normal quantities of salt, (*c*) when a diuresis has been provoked by taking urea by mouth. Gamble (1942, 1944) has set out this aspect of volume control and appears to consider it of general application.

662 m.mol. of urea/l. (3·97 %) may be taken as the maximum to which P.C. concentrated urea in her urine when her blood urea was normal, and the only other osmotic competitors in the urine were the K salts. After a large dose of urea by mouth she produced a urea concentration of 835 m.mol./l. of urine on a normal diet and 860 m.mol. on a salt-free diet (5 and 5·15 % respectively). This was achieved because the diuretic effect of urea reduced

the concentration of Na and K in the urine to a level which permitted the rise in urea to take place without exceeding the osmotic limitations of the kidney. There is nothing in these results to suggest that maximum concentrations of NaCl and urea could be achieved simultaneously (Davies, Haldane & Peskett, 1922; Gilman & Kidd, 1938), but, to see if this could be done, the subjects took both urea and NaCl by mouth on the same day, and P.C.'s results are given in Table 3. There was a large diuresis, and the concentration of Na was well below her maximum for this ion, for the figure of 574 m.mol. was reached in another experiment after taking 15 g. of NaCl (without urea) by mouth. When the salt and urea were taken together the Cl, which is not shown in Table 3, only reached a value of 244 m.equiv./l. against 318 on another occasion, and the concentration of K was low. The urea was well below the level attained on a low salt diet, but the freezing-point of the urine was almost within the range of maximum values, and this is the clue to the whole matter. P.C.'s kidney was incapable of producing simultaneously the concentrations of NaCl and of urea which it could be demonstrated to produce separately. Similar results were obtained on R.H. After 12 g. of NaCl and 20 g. of urea by mouth he passed 1·38 c.c. urine/min. and the concentrations of Cl and of urea in this urine were 1·0 % and 2·86 % respectively. Cl concentrations exceeding 1·3 % were achieved in several of his other experiments; 4 % of urea was found in the urine passed when he was on a salt-free diet, and 5·7 % after taking 25 g. of urea by mouth. The freezing-points varied from $-2{\cdot}2$ to $-2{\cdot}65°$ C.

Fig. 1 shows P.C.'s urine/plasma ratios for urea plotted against her minute volumes. The symbols indicate the nature of the experiments. It will be seen that the U/P ratios fall on or about the smooth curve which has been drawn on the figure, and that this was so whatever the nature of the experiment, and even when urea itself was the agent provoking the diuresis. In other words, whether the minute volume was being fixed by the needs of the body for water, or by the osmotic limitations of the kidney, the U/P ratio taken up was characteristic of that minute volume and largely independent of everything else. This means that, for any given minute volume, the percentage of urea reabsorbed from the glomerular filtrate was unaffected by the amount filtered off, or by the osmotic pressure within the tubules, and this seems to be a matter of considerable theoretical interest. The results certainly support the view that urea is returned to the plasma by diffusion rather than by tubular activity (Dole, 1943).

It will be noted that, in Fig. 1 after a water diuresis on a control diet, two of the U/P ratios were rather far from the curve. In N.J.'s experiment all the U/P ratios were close to the curve, but in R.H.'s experiments the U/P ratios during a water diuresis were more irregular than in P.C.'s, and the ratios were scattered on both sides of the curve. These irregularities were not

due to the phenomena associated with a sudden rise of minute volume after a period of oliguria (Shannon, 1936; Dole, 1943), and it is suggested that they may have been due to spontaneous variations in the glomerular filtration rate. For suppose 'C' to be the number of c.c. filtered off in the glomeruli per minute, and 'V' the minute volume of the urine, then the U/P ratio for a substance such as inulin will be given by C/V. Owing to the fact that some urea is always reabsorbed in the tubules, the U/P ratio for this substance is not

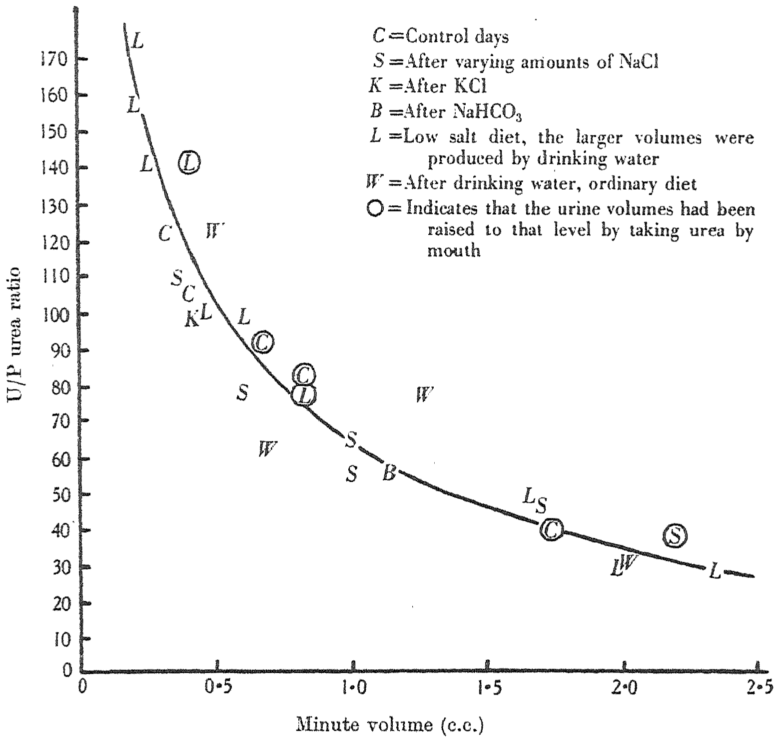

Fig. 1. The effect of various diuretic agents on the U/P urea ratio.

given by C/V, but by C/fV where 'f' is a factor, always greater than 1. In the case of urea, f varies from about 3·6 at low minute volumes to about 1·7 at minute volumes of the order of 2. When the need of the body for water is the sole factor regulating V (as it is in a water diuresis), C, and hence C/fV, can fluctuate without necessarily producing any change in V. If, however, V is being regulated by the osmotic limitations of the kidney, then, since a rise in C must lead to an increase of osmotically active material to be excreted per minute, a rise in C must produce a corresponding rise in V, and this prevents the C/fV ratio going up. Actually, in practice C/fV will fall since the plasma value of the substance under consideration may be

assumed to remain constant. In these experiments the U/P ratios were only far from the curve in a water diuresis produced when the subject was taking an ordinary diet. Similar variations were not found when the subjects were on a low salt diet, and it is suggested that the low salt diet had produced enough shrinkage in the volume of extracellular fluids to curtail for some reason spontaneous variations in the rate of glomerular filtration.

The excretion of NaCl. Table 4 shows data from some of R.H.'s experiments. These illustrate some of the effects of a low-salt diet and of various doses of NaCl by mouth. It will be seen that the serum Na and Cl fell slightly on

TABLE 4. The effect of varying the intake of salt on the serum and urinary concentration

	Serum		Urine				
Experimental procedure	Na mg./100 c.c.	Cl mg./100 c.c.	Min. vol. c.c.	Na mg./100 c.c.	Cl mg./100 c.c.	K mg./100 c.c.	Freezing-point −°C.
Low salt diet	318	355	0·28	74	61	604	2·2
Control diet 1	330	369	0·46	360	690	466	2·67
2	328	367	0·52	610	899	351	2·42
3	330	366	0·63	620	957	348	2·60
8 g. NaCl	333	380	0·86	527	1190	575	2·28
15 g. NaCl	333	386	1·32	695	1330	423	2·12
25 g. NaCl	343	400	2·04	557	1250	330	1·96
30 g. NaCl	347	·403	2·15	590	1160	347	1·85

the low salt diet, and that the serum Na rose only from 330 to 347 mg./100 c.c. even after eating 30 g. of NaCl. The serum Cl rose considerably more—from 367 to 403 mg./100 c.c. after the dose of 30 g.—and this difference in the behaviour of Na and of Cl has been observed in the other two subjects. After being given 35 g. of NaCl, for instance, 11 g. of them intravenously as 3% NaCl, P.C.'s serum Na rose from 335 to 348 mg./100 c.c., the Cl from 379 to 427 mg./100 c.c. Increasing the intake of salt always increased the minute volume of the urine, but an intake of 15 g. of NaCl raised the urinary Cl to a concentration higher than that produced even by the larger doses. This was also noted in other subjects (Fig. 2). It is an old observation (Davies *et al.* 1922) that 1300 mg./100 c.c. is about the maximum concentration of Cl which the kidney can achieve, and these observations fully confirm it. It will next be seen (Table 4) that the concentrations of K were 348–466 mg./100 c.c. urine on the control days, and that the concentration rose on a low salt intake as the minute volume fell. After NaCl, however, the concentrations of K fell off very little, and on some days not at all, in spite of a large increase in the minute volume. It was found in other experiments that, after taking water by mouth, the concentration of K in R.H.'s urine was generally between 80 and 160 mg./100 c.c. when the minute volume was between 0·8 and 2·0 c.c., so that the great increase in K excretion after taking NaCl was no doubt the result of taking NaCl, for others have reported similar findings (McCance & Young, 1944). Finally, it will be noted that the freezing-

point of the urine was appreciably lower on the control diets than it was after taking the large doses of salt. This is better and more convincingly shown by Fig. 2, which gives data from P.C.'s experiments. The concentration of NaCl, and the total osmolar concentrations of the urine, are plotted against the minute volume. It will be seen that, as the intake of salt rose and increased the minute volume of the urine, the concentration of salt remained at or just below the accepted maximum, and the total osmolar concentration of P.C.'s urine fell far below the limits which her kidney achieved on the

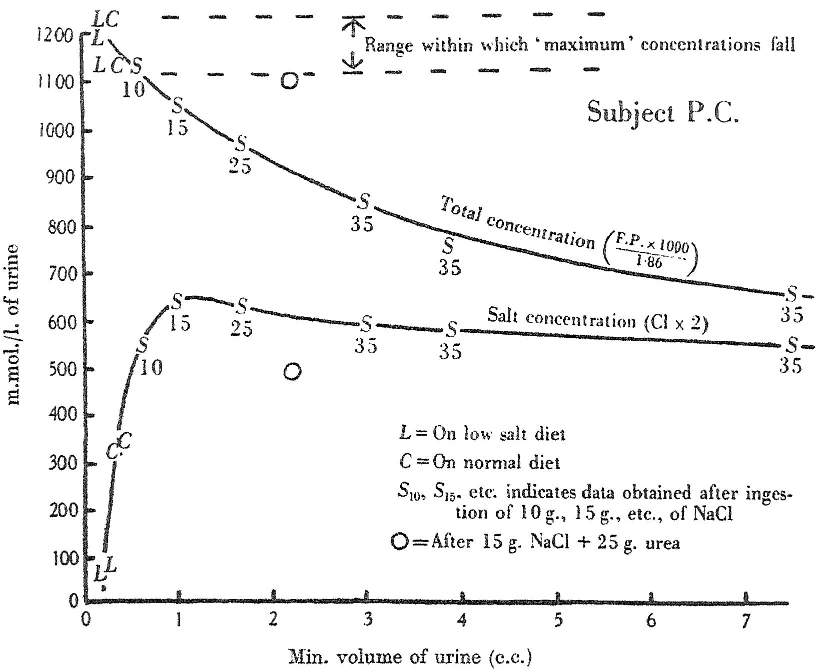

Fig. 2. The effect of a diuresis brought about by salt on the total osmolar concentration of the urine.

control and salt-free diets. In other words, the diuresis after salt was not an osmotic diuresis, and the output of water was being controlled by the forces responsible for the excretion of Na, Cl and possibly K. It is impossible at present to disentangle the separate roles played by the plasma levels, the U/P ratios, the suprarenal cortex and the pituitary gland, and they may all be involved. This fall in the osmolar concentration of the urine is a perfectly logical finding, and was mainly due to the fall in the concentration of urea as the minute volume rose. Further proof that the diuresis after NaCl was in no sense an osmotic diuresis was provided by the experiment in which P.C. took 15 g. of NaCl and 25 g. of urea simultaneously. These results also are given on Fig. 2. At a minute volume of 2·2 c.c. the salt concentration in the

urine was only 490 m.mol./l. so this was not a salt diuresis. The rise in the blood urea, however, to 65 mg./100 c.c. so increased the output of urea that the osmotic concentration of the urine was virtually up to the renal limits, and the diuresis may be regarded as an osmotic one. If a large dose of urea had been taken with the 35 g. of NaCl there is little doubt that the osmolar concentrations of the urines would have been higher than they were. They might even have reached maximum limits at minute volumes of 5 and 6 c.c., but this would have meant that the kidneys' output of osmotic work per minute would have been 14 to 18 times as great as it had been before the doses were taken, and the kidney might not have been able to achieve such a crescendo of power. It may be worth pointing out in this connexion that very little work is involved in maintaining maximum concentrations of Cl in the urine, even when the volumes are high, for the U/P Cl ratio is never above 4. The production of high concentrations of urea, however, at large minute volumes might be difficult to achieve experimentally owing to the large amounts of urea which would have to be introduced into the system, and would involve so much work that the tubules might well be unable to accomplish it in the limited time at their disposal. These matters are being investigated.

The effects of $NaHCO_3$ *and of* K *salts.* All the subjects who took 15 g. NaCl also ingested on another occasion, 21 g. of $NaHCO_3$. These are equimolecular doses. In R.H. and in N.J. the bicarbonate caused a smaller diuresis, 0·7–0·8 c.c./min. as against 1·3 c.c./min. with NaCl, and produced the highest concentration of urinary Na which those persons achieved. It is uncertain

TABLE 5. A comparison of the effects of ingestion of KCl and of NaCl upon the composition of the urine

Subject and dose	Min. vol. c.c.	Freezing-point −°C.	Urea m.mol./l.	Na m.equiv./l.	K m.equiv./l.	Sum Na+K m.equiv./l.	Cl m.equiv./l.
R.H. No dose	0·46	2·67	625	156	119	275	195
5 g. KCl	0·45	2·52	440	137	154	291	296
15 g. NaCl	1·32	2·12	277	302	109	411	375
15 g. KCl	1·27	2·28	288	236	224	460	375
No dose	0·63	2·60	543	270	89	359	270
10 g. KCl	0·62	2·51	417	165	254	419	330
N.J. 5 g. NaCl	0·72	1·97	390	256	54	310	239
5 g. KCl	0·76	1·96	344	210	92	302	260
P.C. Mean of 5 g. and 10 g. NaCl	0·46	2·17	380	212	101	313	222
5 g. KCl	0·46	2·44	382	140	167	307	316

whether this has any general significance for, in P.C., the two salts had almost equal effects. Strong solutions of KCl were found to be very nauseating, even in small doses, but 5–15 g. were taken by each subject to study the reciprocal relationships of Na and K. The results are given in Table 5 where the effects of KCl are compared with those of doses of NaCl which produced similar minute volumes. It will be seen that, in terms of m.equiv./l., (1) the sum of

the Na and K in the urines generally exceeded the Cl, but twice after taking KCl it did not do so, (2) Na and K did replace one another, (3) after taking a K salt the sum of the Na and K equalled or exceeded the sum of the two after taking the Na salt and the concentration of urea was about the same or less. One of the factors limiting the concentration of Na and K is probably the osmotic pressure of the urine, but the present results do not prove this because of the variability of the osmotic maximum.

Discussion

The control of urine volume. The present work makes it clear that in hydropaenia the urine volume may be regulated in at least two ways. The total amount of osmotically active material claiming excretion per minute usually fixes the output of water in mild dehydration, and when the output of NaCl does not exceed 20 mg./min. Chaussin's (1920) results and those of McCance & Young (1944) were obtained under these conditions. The amount of NaCl to be excreted per minute may, however, itself control the output of water, although it is not at present quite clear how. This mechanism becomes effective when the concentration of NaCl in the urine has risen to its maximum, and is most easily demonstrated when the urine volume is over 1 c.c./min. Ambard & Papin (1909) visualized the volumes as being controlled in this way, and Adolph (1923), Baird & Haldane (1922) and Davies *et al.* (1922) were certainly working at times with this mechanism of control. If the output of water is low, it will never be possible to obtain 'maximum' concentrations of NaCl and urea in the same specimen of human urine, because osmotic activity will prevent it. It would probably be easier to obtain really high concentrations of NaCl and urea simultaneously by taking a large quantity of each by mouth. The diuresis which would result would dilute the K salts and other osmotically active constituents of the urine, and allow urea to take their place, and, if the kidney were able to do the necessary amount of osmotic work per minute, the desired result might be obtained. Some of Davies *et al.* (1922) conclusions may be accounted for in this way.

An appreciation of the two ways in which the urine volumes may be regulated in hydropaenia may help to clarify some of the findings in disease. A change over from control by total osmotic activity to control by NaCl concentration probably explains the results reported by Alving & van Slyke (1934) on a patient who was recovering from nephritis.

There are suggestions in the literature (Addis & Shevky, 1922) that healthy persons, deprived of water, produce urines with subnormal specific gravities when their diets contain little salt and protein, but the difference between their averages does not seem to be statistically significant. These results were not in line with those of Addis & Foster (1922), nor did Miller, Price & Longley (1941) confirm them but, in their case, the diets were not low in protein.

The uraemia 'par manque de sel' of the French clinicians (Chabanier & Lobo-Onell, 1934) might be quoted in support, but it is scarcely comparable for all the observations were made on patients, most of them seriously ill and all of them with high blood ureas. The present experiments are of little help in this connexion. P.C. produced urines with freezing-points fully as low as her controls when she was on a diet low in salt (Tables 1 and 6, and Fig. 2) but R.H. and N.J. did not do so quite so convincingly (Table 2). All three might have failed in this if their diets had been low in protein as well as salt, for there may be a maximum U/P urea ratio for each person, and, if there is, some interesting experimental possibilities are opened up. It might, for instance, be possible to demonstrate that this ratio, and not the quantity of osmotically active material, controlled the output of water when the intakes of water, protein and salts were very low. An attempt is being made to do so, and if it succeeds it will be necessary to recognize a third method by which the urine volumes may be controlled in hydropaenia. A consideration of maximum U/P urea ratios leads naturally to the next point.

Chesley's experiments. Chesley's (1938) conclusions have been widely accepted and it should be stated at once that none of the recent experiments directly contradict his results for they have been carried out under somewhat different conditions. They do, however, suggest that Chesley's conclusions require to be carefully rescrutinized, and for the following reasons:

(*a*) Working well within Chesley's range of 'minimal' volumes, it is easy to lower the output of water by reducing the output of NaCl, and this can be counted upon to raise the percentage of urea in the urine. Hence the percentage of urea in urine is not necessarily fixed when the minute volumes are very low.

(*b*) When Black *et al.* (1942) determined the glomerular filtration rates of persons who had lost up to 7·2% of their body weights by dehydration, they found the glomerular filtration rates to be almost or absolutely normal. In a way this is not a direct refutation of Chesley's findings since the subjects studied by Black *et al.* were taking a diet which maintained their urine volumes above Chesley's limiting values. On the other hand, Black's subjects had gone much longer without water than had Chesley's, and one must suppose that glomerular filtration rates vary, if they do vary, with the hydration of the animal rather than with the final volume of the urine.

(*c*) The present results might be taken to support some of Chesley's findings, for, if the volume of the urine is controlled by the output of osmotically active material, a maximum concentration of total solids might be expected at all low minute volumes—and this is what Chesley found.

(*d*) The present results have shown that the osmotic maximum is rather a variable quantity, and it is felt that this should be more carefully watched if Chesley's experiments were being repeated. P.C., for instance, provided

four specimens of urine during her two low salt regimens. The minute volumes, freezing-points, urea concentrations and clearances are given in Table 6. With the freezing-points in the table it is easy to see that variations in the urea

TABLE 6. Variations in the freezing-point and percentage of urea in the urine with no change in the minute volume

Min. vol.	Freezing-point	Urea	Urea clearances (UV/P)
c.c.	$-$°C.	%	c.c.
0·24	2·10	3·25	34
0·24	2·23	3·90	42
0·22	2·37	3·94	35
0·24	2·00	3·20	31

percentages and clearances were mainly due to changes in the osmotic work of the kidney. Had the freezing-points not been determined, and had the minute volumes varied rather than the percentages of urea, these results would have been a direct confirmation of Chesley's conclusions.

SUMMARY

1. After deprivation of water for from 19 to 21 hr., the levels of urea, Na, K and Cl in the serum were constant from day to day within the limits of experimental error, but the concentrations of these substances in the urine, and the freezing-point of the urine varied appreciably.

2. In one subject, large doses of NaCl depressed the level of urea in the serum.

3. The U/P urea ratio depended primarily upon the minute volume of the urine, even when urea itself was the diuretic. Hence, the percentage of urea reabsorbed from the glomerular filtrates must have been independent of the amount filtered off or of the osmotic pressure of the fluid within the tubules.

4. (*a*) Reducing the intake of NaCl lowered the minute volume of the urine and increased the concentration of urea and K in it. (*b*) Taking urea by mouth raised the minute volume of the urine and the percentage of urea in it. The percentage of Na and K fell. In all these experiments the freezing-point of the urine was depressed to the limits which must be regarded as 'maximal', and the flow of urine is considered to have been regulated by the osmotic limitations of the kidney.

5. Administering 25–35 g. of NaCl raised the percentage of NaCl in the urine to its recognized maximum of about 2%, and the volume of the urine to 2–7 c.c./min. In these experiments the freezing-point of the urine indicated that its osmotic pressure was not high, and it is thought that the output of water was being regulated by the amount of NaCl to be excreted per minute.

6. The present experiments explain some of the contradictory findings and conclusions which have been obtained by other workers but they do not support the view that at very low minute volumes the percentage of urea in

the urine is necessarily fixed, or that dehydration lowers the glomerular filtration rate in man.

Miss C. N. Edgecombe was largely responsible for the technical side of this work and I am very grateful to her for this assistance. Much of the enjoyment of this investigation has been due to my good friends, R.H., N.J. and P.C., and I take this opportunity of thanking them for their co-operation. Both Prof. J. B. S. Haldane and M. Grace Eggleton have been kind enough to read the manuscript and to make some very helpful suggestions.

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THE ACTION OF GONADOTROPHIC HORMONE AND OF PITUITARY CORTICOTROPHIC HORMONE ON THE CHOLESTEROL CONTENT OF THE ADRENALS

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It was found, when standardizing gonadotrophic hormone from pregnant-mare serum, that injections of amounts of this hormone sufficient to produce oestrus in young hypophysectomized rats were attended by a considerable mortality. However, none of the experimental animals died if the injected extracts of gonadotrophic hormone had been previously inactivated by heating. The symptoms following lethal injections of the gonadotrophic hormone resembled in many respects those exhibited by rats dying after adrenalectomy. Principal among such symptoms were the anorexia, the stupor with intervening convulsive attacks and the respiratory failure. It was, therefore, decided to study the effect of the gonadotrophic hormone of pregnant-mare serum on the adrenal cortex and particularly on the concentration of cholesterol and of sudanophilic substances in the cortex. The effects of injections of corticotrophic hormone of the anterior pituitary on the cholesterol content of this organ was also investigated, since it is known that this hormone influences the sudanophilic reaction of the adrenal cortex (Reiss, Balint, Oestreicher & Aronson, 1936).

Methods

Male Wistar rats weighing between 30 and 50 g. were used. All injections were made intraperitoneally. Control animals received the same volume of physiological salt solution. The hypophysectomy was made by parapharyngeal approach (Reiss, Druckrey & Hochwald's (1933) modification of Smith's (1930) method) in rats weighing 100–130 g.

The cholesterol content of the adrenals was determined by Bloor's (1922, 1926) method. Since this method was unlikely to yield sufficiently exact figures when applied to the extraction of a single pair of adrenals, it was decided to use the glands of groups of animals treated in the same way. The pooled glands, after careful dissection, were weighed on a torsion balance, ground and extracted with Bloor's mixture. The groups of rats were formed by evenly distributing litters. It will be seen from Table 1 that the cholesterol content of pooled adrenals of groups formed in this manner remains comparable if each group of animals, having received control injections of saline, is killed on different days.

Histological methods. Frozen sections were stained with haematoxylin and Sudan III.

Hormone preparations used. (1) Gonadotrophic preparation: pregnant-mare serum was purified by fractional precipitation with acetone; the crude preparation was re-extracted with 50%

TABLE 1. Cholesterol content of pooled adrenals of control rats, determined on different days after the formation of groups

Days after formation of groups	No. of animals	Cholesterol content of pooled adrenals in mg./100 g. adrenal tissue
1	10	2280
2	10	2320
3	6	2210
4	8	2430
6	10	2370
8	10	2420

alcohol and reprecipitated by increasing the alcohol concentration to 80%. The different preparations used contained 250–300 i.u. gonadotrophic hormone.

(2) Corticotrophic preparation: the crude pituitary extract was prepared by picric acid precipitation of a 0·25% acetic acid extract. This extract was purified by salting out at half-saturation with ammonium sulphate, and finally by isoelectric precipitation at *p*H 4·7. 1 mg. of the preparation used contained between 5 to 20 sudanophobic units (see Reiss *et al.* 1936; Simpson, Evans & Li, 1943).

RESULTS

It will be seen from Table 2 that the cholesterol content of the adrenals of rats killed 24 hr. after administration of gonadotrophic hormone, showed a significant increase when compared with the cholesterol content of the adrenals of a group of control animals. However, in the adrenals of rats injected for

TABLE 2. Cholesterol content of adrenals of male rats treated with gonadotrophic hormone (of pregnant-mare serum)

No. of rats in group	Total amount of gonadotrophic hormone injected (i.u.)	Duration of treatment	No. of injections administered each day	Total cholesterol content in mg./100 g. of the pooled adrenal tissue	Percentage change in cholesterol content
10 untreated controls		—	—	2260	—
6	40	6 hr.	1	3175	+80
6	40	12 hr.	1	2960	+31
6	40	1 day	1	2750	+22
6	40	2 days	1	2320	+ 2
6	40	3 days	1	1980	− 3
10 untreated controls		—	—	2080	—
5	10	7 days	1	787	−62
5	30	7 days	1	464	−78
10 untreated controls		—	—	2630	—
10	40	4 days	1	480	−82
10	40	7 days	1	295	−89

4 days and longer, a marked depletion followed upon the initial increase of the cholesterol content. Table 3 shows that injections of pituitary corticotrophic hormone had the reverse effect on the cholesterol content of the adrenal cortex. The initial effect of the injections of corticotrophic hormone was a decrease in the cholesterol content, while a prolongation of the injections beyond 3 days produced an increase which ranged from +32 to +147% in the various groups of injected animals.

TABLE 3. Cholesterol content of adrenals of male rats treated with corticotrophic hormone

No. of rats in group	Total amount of corticotrophic hormone injected (sudanophobic units)	Duration of treatment	No. of injections administered each day	Total cholesterol content in mg./100 g. of the pooled adrenal tissue	Percentage change in cholesterol content
10 untreated controls		—	—	2940	—
6	8	6 hr.	1	1620	− 45
6	8	12 hr.	2	1850	− 37
6	8	1 day	3	2385	− 19
6	8	2 days	3	3230	+ 10
6	8	3 days	3	3895	+ 32
12 untreated controls		—	—	2600	—
3	14	7 days	3	3600	+ 38
3	28	7 days	3	3880	+ 49
4	21	7 days	3	3430	+ 32
2	42	7 days	3	4325	+ 66
9 untreated controls		—	—	2020	—
8	32	3 days	3	3670	+ 79
6 untreated controls		—	—	2820	—
4	20	3 days	3	4390	+ 56
4	10	3 days	3	4070	+ 44
5 untreated controls		—	—	2630	—
5	1	3 days	3	4920	+ 87
5	2	3 days	3	6230	+137
5	4	3 days	3	4750	+ 81
5	8	3 days	3	5020	+ 91
5	16	3 days	3	6225	+137
5	32	3 days	3	6500	+147

The effect of injections of the pituitary corticotrophic hormone was further investigated in hypophysectomized rats. Such animals show as a rule a diminution of the cholesterol content of the adrenals. The present series of experiments (Table 4) demonstrates that the decrease of the cholesterol content, if it occurs, can be influenced by subsequent injections of corticotrophic hormone. Small doses of this principle are liable to diminish the

TABLE 4. Total cholesterol content of adrenal of (*a*) normal untreated rats, (*b*) hypophysectomized rats, and (*c*) hypophysectomized rats treated with corticotrophic hormone

(Body weight 100–130 g.)

No. of rats in group	Days after hypophysectomy	Total no. of units of corticotrophin injected	Duration of treatment (days)	No. of injections each day	Total cholesterol content in mg./100 g. of the pooled adrenal tissue	Percentage change compared with cholesterol content in normal untreated animals	Percentage change compared with cholesterol content in untreated hypophysectomized animals
8 unoperated and untreated controls				—	2445	—	—
7	17–18	—	—	—	1010	−58	—
2	18	$\frac{1}{2}$	7	2	502	−79	−50
4	14–18	1	7	2	708	−71	−21
3	17	2	7	2	995	−59	− 1
2	17	4	7	2	1350	−45	+24
3	16	8	7	2	1932	−21	+91

Fig. 1. After 7 days' treatment with 70 i.u. (total dose) of gonadotrophic hormone.

Fig. 2. Untreated control.

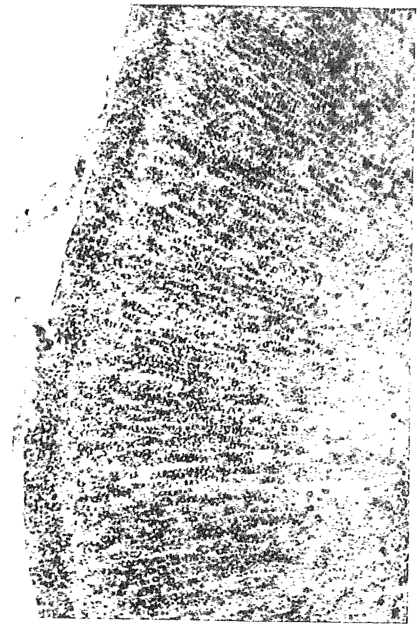

Fig. 3. After 7 days' treatment with 28 sudanophobic units (total dose) of corticotrophic hormone.

Adrenals of 50 g. male litter-mates. Frozen sections. Haematoxylin—Sudan III. ×80.

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cholesterol content even further. However, larger doses of the corticotrophic hormone restored the cholesterol content, with the result that, as shown in Table 4, the values for normal untreated animals were approached.

Histological changes. Changes in the lipid content of the adrenals were also investigated by histological methods (see Pl. 1). It was found that the adrenals of rats, which for 7 days had been injected with gonadotrophic hormone, showed an almost complete loss of sudanophilic granules in the fasciculate and the reticulate zones. The agreement between these findings and the changes in the cholesterol content will be noted. The adrenals of rats injected with corticotrophic hormone for 7 days, showed a marked increase of sudanophilic granules compared with adrenals of a control litter mate. A comparison between these findings and the results of the cholesterol determinations (Tables 2 and 3) shows good agreement between the histological and the biochemical data.

Discussion

The results presented show a diphasic effect of both the corticotrophic and the gonadotrophic hormones on the cholesterol content of the adrenal cortex. The results observed with injection of corticotrophic hormone are in full agreement with those of Sayers, Sayers, White & Long (1943).

The antagonistic action of the gonadotrophic and corticotrophic hormones has also been shown to apply to the blood (Reiss & Langendorf, 1929; Reiss, 1930) for the cholesterol content has been observed to increase after injection of gonadotrophic hormone and to decrease after injection of the corticotrophic hormone.

It seems likely that changes of the cholesterol content of the adrenal cortex reflect changes in the production of the cortical steroid hormones. For instance, it has been shown that changes in the cholesterol metabolism of the adrenal cortex are connected with changes in the production and excretion of 17-ketosteroid hormones. Hemphill, Macleod & Reiss (1942) found that a diminished ketosteroid excretion in certain mental patients could be considerably increased by treatment with corticotrophic hormone. More recent investigations showed, on the other hand, that administration of gonadotrophic hormone decreased the ketosteroid excretion in patients with primary excessive ketosteroid excretion.

Variations in the sudanophilic substances of the adrenal cortex have previously been studied by Reiss *et al.* (1936). It was found that in the hypophysectomized rat, the sudanophilic granules became larger and tended to form clumps; and it was assumed that, in view of the diminished hormone production, this phenomenon was not concerned with the hormone production. When hypophysectomized rats are treated with corticotrophic hormone the earliest change noted is the fragmentation and uniform dispersal of the coarse clumped granules, followed by the gradual elimination of the sudanophobic

zone by the deposition of sudanophilic granules in the zona fasciculata. It will be remembered in this connexion that the cholesterol content of the adrenal cortex of hypophysectomized rats was also shown to increase, provided that a sufficiently large dose of corticotrophic hormone had been administered.

Weaver & Nelson (1943), who recently investigated changes in the birefringent material of the adrenal cortex, obtained essentially similar results.

The histological changes seen in the adrenal cortex after gonadotrophic hormone had been injected for 7 days, are probably due to a mobilization of testosterone or androsterone. This assumption is supported by the findings of Hall & Korenchevsky (1938) who achieved a nearly analogous decrease of sudanophilic lipins after 4–7 weeks' daily injection of small doses (0·1–0·9 mg.) of androsterone or testosterone to castrated male rats.

Summary

1. Injections of corticotrophic hormone of the pituitary anterior lobe and of the gonadotrophic hormone of pregnant-mare serum have an antagonistic influence on the cholesterol metabolism of the adrenal cortex.

2. Gonadotrophic hormone decreases the cholesterol content after a preliminary increase, corticotrophic hormone increases the cholesterol content after a preliminary decrease.

3. The probable connexion of these changes with the production of adrenal cortical hormone and of the 17-ketosteroid excretion are discussed.

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EFFECT OF INTENSITY OF ILLUMINATION ON THE MATCHING OF SOLUTIONS OF CARBOXYHAEMOGLOBIN

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Haldane (1900–1), in the description of his method for determining the amount of haemoglobin in blood, stated that the depth of colour of the unknown and of the standard solutions could be equally well matched in daylight or in artificial light. Haldane mentioned in his paper various factors which are important for accuracy. There was one, however, which he did not mention, namely, light intensity. It is the object of this paper to deal with this factor.

Apparatus

A number of ungraduated haemoglobinometer tubes was obtained, and the mean diameter of each was calculated by the method in which the weight and height of a column of mercury are measured. The mean internal bore was found to vary between 6·61 and 7·02 mm. Two tubes were chosen which differed by 0·04 mm. in internal bore. These were used in all the subsequent experiments, the same tube being always used as the standard.

Solutions of carboxyhaemoglobin were prepared from defibrinated animal blood as follows. Blood (4 ml.) was haemolysed in water (about 50 ml.), the haemoglobin being converted into carboxyhaemoglobin by means of coal gas. The resulting solution was diluted to 200 ml. with water and filtered. The filtrate (stock solution) was a 2 : 100 dilution of blood. 5 ml. of this solution were pipetted into each of five test-tubes labelled $+4$, $+2$, ± 0, -2 and -4 %. Distilled water was added in turn to each tube, 7·0 ml. in tube $+4$ %, 7·25 ml. in tube $+2$ %, 7·5 ml. in tube ± 0 %, and so on throughout the series. In this way a series of solutions of carboxyhaemoglobin was prepared, the concentration in successive tubes in the series differing by 2 %. If the haemoglobin content of the blood sample was not 100 % on Haldane's scale, the amount of blood used to make up the stock solution was adjusted, so that when the stock solution was diluted with an equal volume of water it matched the Haldane standard.

The experiments were conducted in a blacked out passage. The source of light was mounted inside a large wooden box whose inner surface was painted black. In order to obtain the different intensities of illumination required, different arrangements were employed as follows:

0·025 *and* 0·1 *f.c.* A 75 W., 250 V. lamp was used as the source of light. The front of the box was covered by cardboard in which a hole 4 in. in diameter was cut opposite the electric-light bulb. The issuing beam of light was directed on to a hole cut in thick black material stretched across a doorway. The intensity of light at an arbitrarily chosen point on the side of the curtain away from the source of light was measured by means of a Lightometer. The hole in the curtain was slowly enlarged until there was no further increase in the intensity of illumination at the chosen point.

A ground glass screen 10 in. sq. was placed in the cone of light issuing from the aperture in the curtain and the comparisons made by viewing the tubes against this evenly illuminated surface.

1–100 *f.c.* A 500 W., 250 V. lamp was substituted for the 75 W. lamp referred to above.

500 *and* 2000 *f.c.* The front of the box was removed so that the ground glass screen could be placed near enough to the 500 W. lamp to give the desired illumination. Under these conditions, however, the ground glass screen was not evenly illuminated. The comparisons were made by viewing the tubes against the area of maximum illumination.

As the Lightometer scale did not cover the extremes of illumination employed, these were calculated by means of the inverse square law from data derived from known illuminations which were within the range of the instrument.

Plan of experiment

Before an experiment was begun, time was allowed for the eyes of the observer to become adapted to the conditions of illumination. The experiment was carried out by two people. 'The experimenter' filled one tube with the ±0 % solution which was used and retained by 'the observer' as the standard throughout the experiment. The experimenter filled the other tube with one of the solutions of carboxyhaemoglobin mentioned above and presented it to 'the observer' who reported whether the solution of unknown strength matched or was stronger or weaker than the standard.

Each experiment consisted of three comparisons of the standard with the +4, +2, ±0, −2 and −4 % strengths of carboxyhaemoglobin, that is, fifteen comparisons in all. The order of presentation of the unknown solutions was determined by ballot and the observer was unaware of his errors until the end of the experiment. Table 1 gives the detailed results of an experiment chosen at random.

Table 1. Record of an experiment. S = strong; M = match; W = weak. These were 'the observer's' observations of the relative strengths of the unknown solutions presented to him as compared with the standard. The suffixes indicate the order of presentation of the solutions of unknown strength to the observer.

29 November 1944. 50 f.c. Observer: F.M.G.S.

Strength of unknown compared with the standard %	Replies of observer		
+4	S_1	S_3	S_6
+2	M_5	S_{14}	M_{15}
±0 (standard)	M_4	W_8	M_{10}
−2	W_2	W_7	W_{11}
−4	W_9	W_{12}	W_{13}

Number of errors = 3.

Before this series of experiments was begun, a large number of comparisons was made, when the unknown tube was viewed first on the right and then on the left of the standard. It was observed that there were fewer errors in the observations when reliance was placed on the comparison made with the

unknown on the left of the standard. In all the experiments referred to in this paper the decision, i.e. a match, too strong, or too weak, was arrived at mainly as a result of viewing the unknown on the left of the standard. The comparison with the unknown on the right of the standard was used only as a guide, or when the 'left-sided comparison' seemed uncertain.

Results

Ten experiments were performed at each intensity of illumination, five by F.M.G.S. and five by J.L.D. Curve *A* (Fig. 1) shows the relation between the intensity of illumination and the average number of incorrect observations in each experiment, the results of both observers being pooled.

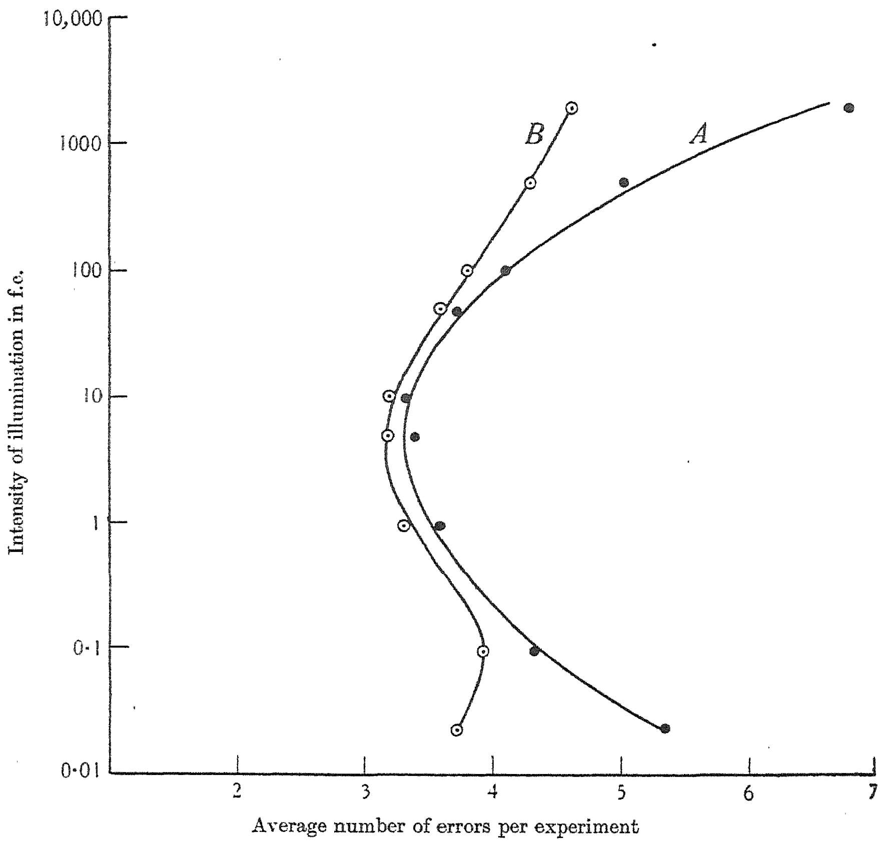

Fig. 1.

The fewest errors were made at an intensity of illumination in the region of 5–10 f.c., but even at 1 and at 50 f.c. the accuracy of comparison was much the same. As the intensity of illumination increased to 2000 f.c. or

diminished to 0·025 f.c., however, the ability to discriminate between the tints of the unknown and standard was continually diminished.

Curve *B* gives additional information as to the accuracy of comparison. It shows the relation between the intensity of illumination and the average number of errors in each experiment for the three concentrations of carboxyhaemoglobin labelled +2, ±0 and −2 %. By considering the curves *A* and *B* together, it may be deduced that at intensities of illumination between 5 and 50 f.c. nearly all the errors of matching occurred when the difference in concentration between the unknown and standard solutions was 2 or 0 %. At 10 and 50 f.c., for example, only once in ten experiments was the +4 or the −4 % concentration of carboxyhaemoglobin incorrectly compared with the standard. This represented one error in 60 comparisons, because each unknown was compared 30 times with the standard in the course of the ten experiments. At intensities of illumination below 1 and above 50 f.c. the curves *A* and *B* diverge, which means that it was more difficult to distinguish between the standard and the +4 or −4 % solutions of carboxyhaemoglobin at these illuminations.

TABLE 2. Each unknown strength of carboxyhaemoglobin was compared thirty times with the standard. The number of errors, expressed as a percentage to the nearest integer, made in distinguishing between the unknown and the standard is recorded for different intensities of illumination.

Strength of unknown carboxyhaemoglobin solution compared with the standard	Percentage of incorrect observations at				
%	0·025 f.c.	1 f.c.	10 f.c.	100 f.c.	2000 f.c.
+4	40	7	3	3	40
+2	53	37	33	33	30
±0 (standard)	57	43	47	47	67
−2	13	30	27	47	60
−4	13	3	0	7	30

Detailed results for some of the intensities of illumination used are given in Table 2. This shows that at 1, 10, and 100 f.c. the +4 and −4 % strengths were nearly always reported correctly as 'strong' and 'weak' respectively. It was as difficult at these illuminations to distinguish the +2 % strength from the standard as it was to distinguish the −2 % strength from the standard. At 0·025 f.c. it appeared to be more difficult to distinguish between the +2 % concentration and the standard than between the −2 % concentration and the standard. This effect is being further investigated. At 2000 f.c. there was considerable difficulty in distinguishing either the +4 or −4 % concentration from the standard solution.

Summary

In this paper the importance of light intensity in haemoglobin determinations using the Haldane method has been investigated. It has been found that the greatest accuracy is obtained at light intensities of 5–10 f.c. Intensities up to 2000 f.c. and down to 0·025 f.c. have been tested. At both high and low light intensities the accuracy is less than that obtained at 5–10 f.c. This is important because instructions are sometimes given to match the solutions when the tubes are viewed against the sky. The illumination in such a case would probably be too bright for the optimum accuracy to be obtained.

We are indebted to Prof. H. Hartridge for suggesting the subject of this work and for his advice, and to Mr Stanley for the loan of a Lightometer.

REFERENCE

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SUGGESTIONS TO AUTHORS

By The Editorial Board

Authors are asked to aim at writing papers as briefly as possible. Brevity, as a rule, accelerates publication, reduces editorial work and the cost of printing, and, lastly, lightens the burden of the reader. Errors from excessive brevity are more easily repaired than those from prolixity. It is important to remember that a reader's impression of a paper is often influenced by its literary style, and in his own interests the author should take pains about his style in order to convey his meaning to his readers. Care should be used in the choice of appropriate words and their place in the sentence, as well as in the sequence and linkage of sentences. A. P. Herbert's *What a Word!* and H. W. Fowler's *Modern English Usage* may here be found helpful. Spellings should follow the *Oxford Dictionary* and Collins's *Authors' and Printers' Dictionary*. Roman and italic words and contractions, both English and foreign, are given in the latter book. (But cf. p. 231.)

Frequent repetition of the first person should be avoided. It is often better, for example, to say 'The following experiments were performed' than 'I performed the following experiments'. The editorial 'we' should be avoided.

Certain principles are to be observed in planning a paper. Except in special circumstances the paper should be subdivided in the following manner:

(1) The object of the research;
(2) Methods used;
(3) Results;
(4) Discussion and inferences;
(5) Summary;
(6) Acknowledgements;
(7) References.

(1) In stating the object of the research the author should refer to previous work only if it has a direct bearing on the subject of the paper, and as a rule a complete historical review is not necessary.

(2) Methods need description once only. Sufficient data should be given to allow the work to be repeated by others.

(3) As a rule the results are best given in words. Large tables of numerical figures alone, from which the reader himself is expected to draw conclusions, should be avoided. It is well recognized, however, that one or a few schematic graphs do often make easier the understanding of the author's results. Accounts of experiments which are essentially similar in nature and result to the leading ones should not be given in detail, though it is well to mention the performance of them. The precision to be attached to numerical values should be given when possible (see p. 225).

(4) A discussion follows the statement of results and is kept separate from it. In this section clarity of expression is all-important.

(5) The summary should be arranged in short numbered paragraphs and should not be longer than one-twentieth of the text.

It is worth remembering that the summary may be used, as it stands, by contributors to abstracting journals. It should, therefore, contain a succinct account of the problem in hand, the method, results and conclusions.

DETAIL

Papers in recent numbers of *The Journal of Physiology* should be studied both for the general manner of presentation and for detail.

General. Papers sent for publication must be typewritten, with double spacing and reasonable margins, on sheets of uniform size, e.g. all foolscap or all quarto. They should be carefully corrected and as nearly ready for press as possible before submission. Insertions should be few and legible.

Papers should be packed flat.

Any matter to be printed in a smaller size of type than usual is to be marked by a vertical line in the left-hand margin. The following conventional marks may be used to guide the printer:

One underline indicates *italics* which, if used freely in the text, defeat their own object. Letter spacing as a means of emphasis will not be used.
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Title. The title is to be typed on a separate sheet. The name of the author follows, together with any necessary description, e.g. *Beit Memorial Research Fellow.* When there are several authors their names should be in alphabetical order. The name of the laboratory and its address complete the title.

A short title also is needed for the index of the volume. It must not exceed four or five words and will be printed in capitals at the head of the right-hand pages in the *Journal.* Authors are asked to supply the short title.

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1.	PART I	(Capitals)
2.	RESULTS	(Small capitals)
3.	A. The normal intestine	(Caps. and l.c. Roman)
4.	(I) *The action of drugs*	(Italics, centre)
5.	(a) *Histamine*	(Italics, full out)
6.	(1) *Peristalsis.* This type of movement......	(Italics, indent)

The usual headings required are 2, 4, 5 and 6. Only exceptionally are 1 and 3 used. It is often convenient to precede each subheading in the manuscript with a letter or number characteristic of the grade, as indicated above, e.g. (A), (I) etc. Such letters and numbers will not generally appear in print but they are of assistance to the editors and printers. Recent numbers of the Journal should be consulted for further examples of the customary use of headings. Note that no full stops are required after headings except after those in grade 6. A paragraph starting after a central heading is not indented.

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Kymograph records should include appropriate scales as far as possible. Large black areas, which are not needed, should be cut away. Letters and numbers on kymograph records are acceptable and are not altered by the engraver, though they must be large enough to withstand photographic reduction. If they are not, they are best blacked out.

Half-tone reproductions lose some of their contrast and definition from the original. Hence the original should be excellent in these respects, or if not, it may be strengthened by an intensifier or copied by means of process plates

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Year of publication in brackets. When the publication of a volume is spread over more than one year, give, if possible, the publication year of the article in question. If several papers by the same author in one year are cited, *a*, *b*, *c*, etc., are placed after the year of publication.

Journal's title, abbreviated in accordance with the *World List of Scientific Periodicals*, and underlined to indicate italics.

Volume number in arabic numerals underlined with a wavy line to indicate black type, without prefix 'vol.'

The number of the first page in arabic numerals, without prefix 'p.'

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Langley, J. N. (1919*a*). *J. Physiol.* **53**, 120.

Langley, J. N. (1919*b*). *J. Physiol.* **53**, 147.

Lucas, K. & Mines, G. R. (1907). *J. Physiol.* **36**, 334.

When reference is made to a book, the title should be underlined; the edition, volume number (vol.) and page (p. or pp.) should be given, followed by the town of origin and the publisher, thus:

Starling, E. H. (1915). *Principles of Human Physiology*, 2nd ed. p. 184. London: Churchill.

When particular parts, fascicles or numbers, with roman numerals or other system not here used, need reference, ordinary abbrevations should be used, thus:

Graefe, A. & Saemisch, T. (1874). *Handbuch der gesammten Augenheilkunde*, Band **2**, Teil 2, p. 547. Leipzig: Engelmann.

References to authors quoted at second-hand should appear in the list of references in the following form:

Hering, E. (1915). *v. Graefes Arch. Ophthal.* **90**, 1. Cited by Hecht, S. in *Physiol. Rev.* 1937, **17**, 239.

or

McGrady, E. (1944). *J. Tenn. Acad. Sci.* **19**, 240. Cited in *Brit. Abstr.* A III, 1945, 150.

In references to publications produced annually or at less frequent intervals, and where the author's work covers a specific period, the year of issue of the journal should follow the author's name, and the date of the particular paper should be placed after the title of the journal:

Cole, L. B. (1927). *St Thom. Hosp. Rep.* 1925, **49**, 255.

In the list of references attention should be paid to the customary punctuation.

In the text, references are made by giving in brackets the name of the author and the year of publication, e.g. (Taylor, 1931), except when the author's name

is part of the sentence, e.g. 'Taylor (1931) showed that...'. When a paper written by two authors is quoted, both names are given, the ampersand (&) being used in place of and, e.g. Taylor & Taylor (1931). If there are more than two authors all names should be given when cited for the first time, and thereafter the first name only, adding '*et al.*'

Quotations. In quotations use single quotes, e.g. ' ' and not " ".

Footnotes. Footnotes are expensive and tiresome and are best avoided.

Proofs. Proofs are sent to authors in order that they may correct mistakes in printing and not that they may add new material. If they do add new material there is a great risk of delay in publication; and increased printing charges to the Society are inevitable. Excessive alteration may have to be disallowed. It is therefore important that the original typescript should be as free as possible from errors. If, however, alteration in the middle of a paragraph is unavoidable, the insertion should be arranged to fit neatly without upsetting unoffending lines of neighbouring type. Should some amplification or correction become necessary when the paper has already reached proof stage, it is often better to add a note at the end of the paper, headed 'Note added in proof', rather than to make extensive insertions and alterations in the text.

When postal delays are likely to occur, authors residing in other countries may nominate someone in Great Britain who would be willing to correct the proofs. Papers from such contributors should be accompanied by a statement of the number of reprints required.

MATHEMATICAL NOTATION

The rules laid down for mathematical notation and numerals in *Proceedings of the Royal Society*, A, 1909, **82**, 14, should be followed.

A numeral should not, in general, be used to begin a sentence, but numerals may be used in the text as abbreviations for specific quantities. It is, however, permissible to use numerals at the beginning of a sentence when they refer to a number of units which are already expressed in an abbreviated form, e.g. '10 c.c.', but 'Twenty animals'; not Ten c.c. nor 20 animals. Numbers above 100 are preferably given as numerals in all circumstances, but it is better not to begin a sentence with such a number.

Fractions and ratios should be written 1/100, not $\frac{1}{100}$. Such common fractions as $\frac{1}{2}$, $\frac{1}{4}$, etc., are, however, permissible. As far as possible, the decimal system should be used to indicate fractions. The decimal point must always be preceded by a figure, if necessary 0 being prefixed, e.g. 0·1 must be written, never ·1. In a column of numbers containing decimal fractions the decimal points should always be arranged in the same vertical line.

Indication of the degree of precision to be attached to numerical results. In general, the number of digits in a numerical result is taken to indicate its probable accuracy. When a value given is the arithmetic mean of a number of

separate determinations, the approximate amount of variation of the individual determinations should be stated. The mean alone without the variation is of no more use to the reader than a single observation. Where space permits, it is desirable to give all the observations in full, but authors who can estimate the error should do so. An estimate of the standard error of the mean is the best index of accuracy of the mean which can be calculated from a set of parallel observations. Its use is always justified when the use of the mean is justified, even if this mean is calculated from only two observations. If, for instance, the value 263 is given to a quantity the results should be stated on the first occasion in the form $263 \pm 0{\cdot}5$ (S.E. of mean of 10 observations), subsequently abbreviated as $263 \pm 0{\cdot}5$ (10).

If graphs are drawn through points each of which represents the mean of several observations, the error may be shown by rectangles enclosing the range corresponding to $\pm$ the standard error of each of the variables plotted. Often the error of one variable is negligible, so that the rectangle becomes a straight line.

CHEMICAL NOMENCLATURE

In general, symbols, abbreviations and spellings should be those adopted by the Chemical Society, the Bureau of Abstracts and the Biochemical Society. The next two paragraphs are quoted from 'Directions to Contributors' published in the *Biochemical Journal.*

(1) *Chemical formulae* should be written as far as possible on a single horizontal line. With inorganic substances, formulae may be used, at the discretion of the editors, wherever their interpretation is clear. With salts, it must be stated whether or not anhydrous material is used, e.g. anhyd. $CuSO_4$, or which of the different crystalline forms is indicated, e.g. $CuSO_4.5H_2O$, or $CuSO_4.H_2O$.

(2) *Solutions* of common acids, bases and salts are preferably defined in terms of normality (N) or molarity (M), e.g. N-HCl; 0·1 M-NaH_2PO_4. The term '%' must be used in its correct sense, i.e. g./100 g. of solution. 10% HCl means 10 g. of hydrogen chloride in 100 g. of aqueous solution, and should never be used to indicate a tenfold dilution of laboratory concentrated hydrochloric acid. For 'per cent by volume', i.e. c.c./100 c.c., the term '% (v/v)' may be employed. To indicate that a given weight of substance is contained in 100 c.c. of solution, the term '% (w/v)' (weight per volume) may be used.

(3) Such terms as acid sodium phosphate should be avoided. Salts of acids containing more than one replaceable hydrogen atom should be described by naming the actual kations, e.g. sodium dihydrogen phosphate and disodium hydrogen phosphate.

(4) The termination *ine*, and not *in*, should be reserved for bases, e.g. adrenaline, choline, atropine, etc. The termination *in* is used for certain neutral substances, e.g. dextrin, pepsin, albumin, etc.

(5) Most of the amino-acids follow rule (4): e.g. leucine, glycine, thyroxine,

etc. Tryptophan does not have a final *e*, since *ane* is the termination reserved for saturated hydrocarbons, e.g. methane.

(6) All alcohols should have names ending in *ol* not *ite* or *ine*, e.g. glycerol (when used as a chemical term), mannitol, etc. Compounds which are not alcohols, but which are commonly given names ending in *ol*, should be spelt with a terminal *e*, e.g. indole, iminazole, pyrrole, etc. Alcohols should be given their specific names, e.g. methanol, ethanol, etc.

(7) Fatty substances are lipids (adjective lipoid). Lipin is only used as a suffix, e.g. phospholipin.

Pharmacopoeial, i.e. official, names should be given when available and proprietary names avoided if possible. When proprietary preparations are used, the actual chemical composition should be given in brackets after the first use of the name. The names of non-pharmacopoeial drugs can usually be found in *The British Pharmaceutical Codex* and *The Extra Pharmacopoeia*. When the name of a new drug is first used, the brand should be stated.

BIOLOGICAL NOMENCLATURE

When the true form of Latin names of animals or plants is used, it should begin with a capital letter. When used as an adjective, or in any other form, the word should begin with a lower-case letter, e.g. Protozoa, but protozoan; Mammalia, but mammals and mammalian.

Generic and specific names should be underlined (i.e. in italics). The specific epithet or trivial name should commence with a small letter.

Names of muscles, bones, etc., should be in roman type (i.e. not italics). Adrenal is preferable to suprarenal. Care should be taken in the use of such words as dorsal, ventral, caudal, anterior and posterior. The terms caudad, craniad, dorsad and ventrad may be used to indicate 'towards the tail', 'towards the head', etc.

Rate should not be confused with frequency; pulse-rate, heart-rate and respiration-rate are permissible as technical terms, meaning frequency of the pulse, frequency of heart beat, and frequency of respiratory movements respectively. Respiration rate can then be used in connexion with the oxygen consumption of tissues. 'Breathing' or 'respiratory movements' should be used rather than 'respiration', the latter term being reserved for the respiratory exchanges of tissues or organs.

Ringer's solution and its variants. The composition of the particular solution used should always be given. Such terms as 'normal saline' and 'saline' should be avoided.

The suffix -trophic (not tropic) should be used in such words as corticotrophic.

DECIMAL SYSTEM

The decimal system of the *Institut Internationale de Documentation* has been adopted by *The Journal of Physiology* in common with other journals, in order to provide a subject classification of papers and reprints.

PROOF CORRECTION

[Proof]

1.	s.c.	Results
2.	indent	Most of the experiments in the present
3.	9/	invəstigation were carried out with
4.	,/	cocaine; and the results to be described
5.	below/	~~bloew~~ were obtained with this unless
6.	#/	specialmention is made.
7.	Ital/	Magnitude of action potential.
8.	δ/	(1) Conduction along the distal ~~non~~ non-
		narcotised segment. In view of the
9.	Rom/	finding of *Woronzow* that the size
10.	tr/	the of electric response suffers decrement
11.	tr/	along the distal region most beyond the
12.	tr/	depressed area, it is intreesting, first
13.	see/	of all, to ~~find~~ whether the same
14	λ the	would occur under present experimental
15.	/run on	conditions.
16.	DD/caps	For this purpose dd leads were first
17.	L/	employed. Records of propagated
18.	stet/	disturbances were taken ~~both~~ before and
19.	×/	at varying intervals during narcosis and
20.	¶/	recovery. With ND leads diphasic re-
21.	l.c/	cords were obtained in which the POTENTIAL
22.	w.f./	variations appearing in the narcotised
23.	-/	and non narcotised distal regions are
24.	=/	simultaneously represented in the two
		phases of each response, the first phase
25.		being derived from the form er and the
26.	⊙/	second from the latter;

Explanation of marks.

1. To change to small capitals.
2. When a word is too far to the left.
3. When a letter is inverted.
4. Substitution of comma for another point or letter. Similarly for colon and semi-colon.
5. When letters in a word are misplaced.
6. Insertion of a space.
7. When italics should be used.
8. When a letter or word is to be omitted.
9. When a word is to be changed to roman type.

10, 11, 12. Methods of marking transposition. When several words are badly mixed up they may be numbered, the usual mark being placed in the margin.

13. Substitution of one word for another.

[The same corrected]

RESULTS

Most of the experiments in the present investigation were carried out with cocaine, and the results to be described below were obtained with this unless special mention is made.

Magnitude of action potential.

(1) *Conduction along the distal non-narcotised segment.* In view of the finding of Woronzow that the size of the electric response suffers most decrement along the distal region beyond the depressed area, it is interesting, first of all, to see whether the same would occur under the present experimental conditions. For this purpose DD leads were first employed. Records of propagated disturbances were taken both before and at varying intervals during narcosis and recovery.

With ND leads diphasic records were obtained in which the potential variations appearing in the narcotised and non-narcotised distal regions are simultaneously represented in the two phases of each response, the first phase being derived from the former and the second from the latter.

Explanation of marks (cont.).

14. When a letter or word is to be inserted.
15. When a paragraph occurs wrongly.
16. Substitution of capitals for small letters.
17. To reduce spacing.
18. When words crossed out are to remain.
19. When letters appear imperfectly.
20. Mark for a paragraph.
21. To change capitals to small letters.
22. When a letter from a wrong fount has been used.
23. To insert a hyphen.
24. When letters or words are out of line.
25. When letters are to be brought close together.
26. Insertion of full stop when omitted or replaced by another point.

SOME RECOMMENDED ABBREVIATIONS AND SYMBOLS, CHIEFLY FOR USE IN TABLES AND FIGURES

In using abbreviations, no difference is made between singular and plural, e.g. 1 min. and 40 min. or 24 hr., except in cases noted in list below.

Absolute	abs.
Alternating current	a.c.
Ampere	amp.
Ampere, with qualifying prefix or suffix only, e.g. milli-ampere mA.	A.
Analytical standard of purity	A.R.
Ångström unit	Å.
Ante-meridiem	a.m.
Approximately, e.g. 50 c.c. approx.	approx.
Atmosphere	atm.
Atomic weight	at.wt.
Blood pressure	B.P.
Boiling-point	b.p.
British Pharmacopoeia	B.P.
British Thermal Unit	B.TH.U.
Calculated (in table headings)	calc.
Calorie, large, kilogram-calorie	kg.cal.
Calorie, small, gram-calorie	cal.
Candle-power	c.p.
Centimetre, square centimetre	cm., cm.^{2}
Centimetre gram second	c.g.s.
Centimetres per second	cm./sec.
Central Nervous System	C.N.S.
Cerebrospinal fluid	c.s.f.
Coefficient	coeff.
Concentrated	conc.
Constant	const.
Corrected	corr.
Crystalline or crystallized	cryst.
Cubic, in all cases except c.c.	cu.
Current density	c.d.
Cycles per second	cyc./sec.
Decibel	db.
Degrees, e.g. 15 degrees	15°
Degrees, absolute	° K.
Degrees, centrigrade	° C.
Degrees, Fahrenheit	° F.
Dextrorotatory	*d*-
Direct current	d.c.
Dissociation constant, negative logarithm of	pK
Divalent, trivalent, etc., e.g. divalent iron, etc.	Fe^{II}
Electrocardiogram	e.c.g.
Electroencephalogram	e.e.g.
Electromotive force	e.m.f.
Electroretinogram	e.r.g.
Equivalent (weight)	equiv.
Experiment	exp.
but Experiment 4	Exp. 4 (Exps.)
Farad	F.
Feet per second	ft./sec.
Figure (illustration)	Fig. (Figs.)
Foot, feet (except foot-candle)	ft.
Foot-candle	f.c.
Foot-pound	ft.lb.
Freezing-point	f.p.
Gram	g.
Gram-calorie	cal.
Gram-ion	g.-ion
Gram-molecule	mol.
Gravity, acceleration due to	*g*
Height	ht.
Henry	H.
High frequency	h.f.
Horse-power	h.p.
Hour	hr.
Hydrogen-ion concentration	*c*H or [H+]
Hydrogen-ion concentration, negative logarithm of	pH
Inch	in.
Intensity	*I*
International unit	i.u.
-ion, e.g. calcium-ion, sulphate-ion, etc.	Ca^{++}, SO_4^{--}
Kilo- ($10^3 \times$), e.g. kilogram	k, e.g. kg.
Kilovolt-ampere	kVa.
Kilowatt-hour	kWh.
Laevorotatory	*l*-
Litre	l.
Logarithm	log
Logarithm, when not used as a mathematical symbol	log.
Low frequency	l.f.
Maximum	max.
Megacycles per second	Mcyc./sec.
Megohm	MΩ.
Melting-point	m.p.
Meta-	*m*-
Metre	m.
Metre-candle	m.c.
Micro- ($10^{-6} \times$), e.g. microgram	μ, e.g. μg.
Micromicro- ($10^{-12} \times$)	$\mu\mu$
Micromicron (10^{-12} m.)	$\mu\mu$.
Micron (10^{-6} m.)	μ.
Milli- ($10^{-3} \times$)	m
e.g. milligram	mg.
but millimol	m.mol.
milliequivalent	m.equiv.
Millimicro- ($10^{-9} \times$)	mμ
Millimicron (10^{-9} m.)	mμ.
Millimolar (concentration)	mM
Minimum	min.

Minute	min.
Molar (concentration)	M.
Molar, with chemical formula or name	M-
Molecular, molecule	mol.
Molecular weight	mol.wt.
Normal (concentration)	N.
Normal, with chemical formula or name	N-
Normal temperature and pressure	N.T.P.
Number	no.
but Number 4	No. 4 (Nos.)
Observed (in table headings)	Obs.
Ohm	Ω.
Ortho-	*o*-
Page, pages	p., pp.
Para-	*p*-
Per (e.g. metres per sec.)	/ (e.g. m./sec.)
Per cent	%
Plate (illustration)	Pl.
Post-meridiem	p.m.
Potential difference	p.d.
Pound	lb.
Pounds per square inch	lb./sq.in.
Precipitate (noun)	ppt.
Relative humidity	R.H.
Respiratory quotient	R.Q.
Revolutions per minute	r.p.m.
Röntgen unit	r.
Second (unit of time)	sec.
Secondary	*sec.*-
Solution (table heads only)	Soln.
Specific gravity	sp.gr.
Specific heat	sp.ht.
Square, e.g. square metre	sq., sq.m.
Temperature	temp.
Tertiary	*tert.*-
Vapour density	v.d.
Vapour pressure	v.p.
Velocity	vel.
Volt	V.
Volume	vol.
Watt	W.
Wave-length	λ.
Weight	wt.
Yard	yd.

RECOMMENDED SPELLINGS FOR COMMONLY USED WORDS, ETC.

A priori (Roman type)
Accommodation
Acknowledgement
After-effect
Airtight
Alinement
Analyse
Blood pressure (but blood-pressure readings)
Body weight
Connexion
Co-ordinate
Cotton-wool
Coverslip
Debris
Deflexion
Desiccate
Develop
End-point
Entered
Focused
Fresh water (but fresh-water environment)
Hydrolyse
In situ, in vitro, etc. (Roman type)
Isoelectric
Judgement
Life cycle
Microchemical (but micro-organism)
Occurred
Phlorrhizin
Post-mortem (Roman type)
Re-examine
Reflexion
Re-illuminate
Résumé
Role
Sea water (but sea-water environment)
Stopwatch
Subculture
Subfamily
Subnormal
Un-ionized
Vice versa (Roman type)
Watch-glass
Water-bath
Water-level
Watertight

A LIST OF PERIODICALS AND THEIR ABBREVIATIONS

The abbreviations generally follow those of the World List of Scientific Periodicals (Oxford)

Periodical	Abbreviation
Abhandlungen der Kgl. Sächsischen Gesellschaft (Akademie) der Wissenschaften. Math.-Phys. Kl. Leipzig	*Abh. sächs. Ges. (Akad.) Wiss.*
Acta Brevia Neerlandica de Physiologia, Pharmacologia, Microbiologia, etc.	*Acta Brev. neerl. Physiol.*
Acta medica Scandinavica	*Acta med. Scand.*
Acta obstetrica et gynecologica Scandinavica	*Acta obstet. gynec. Scand.*
Acta ophthalmologica	*Acta ophthal., Kbh.*
Acta oto-laryngolica	*Acta otolaryng., Stockh.*
Acta pathologica et microbiologica Scandinavica	*Acta path. microbiol. Scand.*
Acta physiologica Scandinavica	*Acta physiol. Scand.*
Acta phytochimica, Tokyo	*Acta phytochim., Tokyo*
Acta Scholae Medicinalis Universitatis Imperialis in Kioto	*Acta Sch. med. Univ. Kioto*
Acta Societatis scientiarum Fennicae	*Acta Soc. Sci. fenn.*
Advances in Enzymology	*Advances in Enzymology*
Agricultural Research Council Reports	*Agric. Res. Coun. Rep.*
American Journal of Anatomy	*Amer. J. Anat.*
American Journal of Diseases of Children	*Amer. J. Dis. Child.*
American Journal of Hygiene	*Amer. J. Hyg.*
American Journal of Obstetrics and Gynaecology	*Amer. J. Obstet. Gynaec.*
American Journal of Pathology	*Amer. J. Path.*
American Journal of Pharmacy	*Amer. J. Pharm.*
American Journal of Physiology	*Amer. J. Physiol.*
American Journal of Psychology	*Amer. J. Psychol.*
American Journal of the Medical Sciences	*Amer. J. med. Sci.*
American Journal of Tropical Diseases	*Amer. J. trop. Dis.*
Anatomical Record	*Anat. Rec.*
Anatomischer Anzeiger	*Anat. Anz.*
Angewandte Chemie	*Angew. Chem.*
Animal Breeding Abstracts	*Anim. Breed. Abstr.*
Annalen der Physik	*Ann. Phys., Lpz.*
Annales de chimie (et de Physique)	*Ann. Chim. (Phys.)*
Annales de la Société Royale des sciences médicales et naturelles de Bruxelles	*Ann. Soc. Sci. méd. nat. Brux.*
Annales de l'Institut Pasteur	*Ann. Inst. Pasteur*
Annales de médecine	*Ann. Méd.*
Annales de Physiologie et de physicochimie biologique	*Ann. Physiol. Physicochim. biol.*
Annales de physique	*Ann. Phys., Paris*
Annali d' igiene (sperimentale)	*Ann. Igiene (sper.)*
Annals of Applied Biology	*Ann. appl. Biol.*
Annals of Biochemistry and Experimental Medicine (Indian Institute for Medical Research)	*Ann. Biochem. exp. Med.*
Annals of Internal Medicine	*Ann. intern. Med.*
Annals of Surgery	*Ann. Surg.*
Annual Review of Biochemistry	*Ann. Rev. Biochem.*
Annual Review of Physiology	*Ann. Rev. Physiol.*
Arbeitsphysiologie	*Arbeitsphysiologie*
Archiv der Pharmazie	*Arch. Pharm., Berl.*
Archiv für Anatomie und Pharmakologie	*Arch. Anat. Pharmak.*
Archiv für Anatomie und Physiologie	*Arch. Anat. Physiol., Lpz.*
Archiv für Augenheilkunde	*Arch. Augenheilk.*
Archiv für die gesamte Physiologie	*Pflüg. Arch. ges. Physiol.*
Archiv für Entwicklungsmechanik der Organismen	*Arch. EntwMech. Org.*
Archiv für experimentelle Pathologie und Pharmakologie	*Arch. exp. Path. Pharmak.*
Archiv für experimentelle Zellforschung	*Arch. exp. Zellforsch.*
Archiv für Gynaekologie	*Arch. Gynaek.*
Archiv für Hygiene	*Arch. Hyg., Berl.*
Archiv für mikroskopische Anatomie (und Entwicklungsmechanik)	*Arch. mikr. Anat.*

Periodical	Abbreviation
Archiv für Ophthalmologie	*v. Graefes Arch. Ophthal.*
Archiv für pathologische Anatomie und Physiologie	*Virchows Arch.*
Archiv für Verdauungskrankheiten	*Arch. VerdauKr.*
Archives de biologie	*Arch. Biol., Paris*
Archives de médecine expérimentale et d'anatomie pathologique	*Arch. Méd. exp.*
Archives de physiologie normale et pathologique	*Arch. Physiol. norm. path.*
Archives des sciences biologiques	*Arch. Sci. biol., St Pétersb.*
Archives d'ophtalmologie	*Arch. Ophtal., Paris*
Archives internationales de pharmacodynamie (et de thérapie)	*Arch. int. Pharmacodyn.*
Archives internationales de physiologie	*Arch. int. Physiol.*
Archives italiennes de biologie	*Arch. ital. Biol.*
Archives néerlandaises de physiologie de l'homme et des animaux	*Arch. néerl. Physiol.*
Archives of Biochemistry	*Arch. Biochem.*
Archives of Disease in Childhood	*Arch. Dis. Childh.*
Archives of Internal Medicine	*Arch. intern. Med.*
Archives of Neurology and Psychiatry, Chicago	*Arch. Neurol. Psychiat., Chicago*
Archives of Neurology and Psychiatry, London	*Arch. Neurol. Psychiat., Lond.*
Archives of Ophthalmology	*Arch. Ophthal., N.Y.*
Archives of Pathology and Laboratory Medicine	*Arch. Path. Lab. Med.*
Archives of Surgery, Chicago	*Arch. Surg., Chicago*
Archives of Surgery, London	*Arch. Surg., Lond.*
Archivio di farmacologia sperimentale e scienze affini	*Arch. farmacol. sper.*
Archivio di fisiologia	*Arch. Fisiol.*
Archivio per le scienze mediche	*Arch. Sci. med.*
Atti della R. Accademia dei Lincei (Memorie)	*Mem. Accad. Lincei*
Atti della R. Accademia dei Lincei (Rendiconti)	*R.C. Accad. Lincei*
Atti della R. Accademia delle scienze	*Atti Accad. Torino*
Atti della Società lombarda di scienze mediche e biologiche	*Atti Soc. lombarda Sci. med. biol.*
Atti del R. Istituto veneto di scienze, lettere ed arti	*Atti Ist. veneto*
Australian Journal of Experimental Biology and Medical Science	*Aust. J. exp. Biol. med. Sci.*
Beiträge zur chemischen Physiologie und Pathologie	*Beitr. chem. Physiol. Path.*
Beiträge zur Geburtshilfe und Gynäkologie	*Beitr. Geburtsh. Gynäk.*
Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie	*Beitr. path. Anat.*
Bericht der Deutschen Chemischen Gesellschaft	*Ber. dtsch. chem. Ges.*
Berichte über die gesamte Physiologie und experimentelle Pharmakologie	*Ber. ges. Physiol.*
Berliner klinische Wochenschrift	*Berl. klin. Wschr.*
Bibliothek for læger	*Bibl. Læger*
Biochemical Journal	*Biochem. J.*
Biochemische Zeitschrift	*Biochem. Z.*
Biochimia	*Biochimia*
Biological Abstracts	*Biol. Abstr.*
Biological Bulletin of the Marine Biological Laboratory, Woods Hole	*Biol. Bull. Woods Hole*
Biological Reviews	*Biol. Rev.*
Biological Symposia	*Biol. Symp.*
Biologisches Zentralblatt	*Biol. Zbl.*
Bolletino della Società italiano di biologia sperimentale	*Boll. Soc. ital. Biol. sper.*
Brain	*Brain*
British Abstracts A III—Physiology, Biochemistry, Anatomy	*Brit. Abstr.* A III
British Dental Journal	*Brit. dent. J.*
British Heart Journal	*Brit. Heart J.*
British Journal of Children's Diseases	*Brit. J. Child. Dis.*
British Journal of Experimental Biology	*Brit. J. exp. Biol.*
British Journal of Experimental Pathology	*Brit. J. exp. Path.*
British Journal of Industrial Hygiene	*Brit. J. industr. Hyg.*
British Journal of Ophthalmology	*Brit. J. Ophthal.*
British Journal of Psychology	*Brit. J. Psychol.*
British Journal of Radiology	*Brit. J. Radiol.*
British Medical Bulletin	*Brit. med. Bull.*
British Medical Journal	*Brit. med. J.*

Periodical	Abbreviation
Bulletin biologique de la France et de la Belgique	*Bull. biol.*
Bulletin de Biologie et Médecine Expérimentale de l'U.R.S.S.	*Bull. Biol. Med. exp.*
Bulletin de l'Académie de médecine de Belgique	*Bull. Acad. Méd. Belg.*
Bulletin de l'Académie de médecine	*Bull. Acad. Méd. Paris*
Bulletin de la Société chimique de Paris	*Bull. Soc. chim., Paris*
Bulletin de la Société de chimie biologique	*Bull. Soc. Chim. biol., Paris*
Bulletin et Mémoires de la Société des hôpitaux de Paris	*Bull. Soc. méd. Hôp. Paris*
Bulletin of War Medicine	*Bull. War. Med.*
Bulletin. Société chimique de France	*Bull. Soc. chim. Fr.*
Bureau of Standards Journal of Research	*Bur. Stand. J. Res., Wash.*
Cancer Research	*Cancer Res.*
Carnegie Institution of Washington Publications	*Publ. Carneg. Instn*
Chemical Abstracts	*Chem. Abstr.*
Chemische Zeitschrift	*Chem. Z.*
Chemistry and Industry	*Chem. Ind.*
Chinese Journal of Physiology	*Chin. J. Physiol.*
Chinese Journal of Physiology. Report Series	*Chin. J. Physiol. Rep. Ser.*
Clinical Science	*Clin. Sci.*
Cold Spring Harbor Symposia on Quantitative Biology	*Cold Spr. Harb. Sym. quant. Biol.*
Compte rendu de l'Académie des sciences de l'U.R.S.S.	*C.R. Acad. Sci. U.R.S.S.*
Compte rendu hebdomadaire des séances et mémoires de la Société de biologie	*C.R. Soc. Biol., Paris*
Compte rendu des travaux du Laboratoire Carlsberg	*C.R. Lab. Carlsberg*
Compte rendu hebdomadaire des séances de l'Académie des Sciences	*C.R. Acad. Sci., Paris*
Contributions to Embryology (Publications of the Carnegie Institution)	*Contr. Embryol. Carneg. Instn*
Current Science	*Curr. Sci.*
Dental Record	*Dent. Rec.*
Deutsche medizinische Wochenschrift	*Dtsch. med. Wschr.*
Deutsche Monatsschrift für Zahnheilkunde	*Dtsch. Mschr. Zahnheilk.*
Deutsche Zeitschrift für Nervenheilkunde	*Dtsch. Z. Nervenheilk.*
Documenta Ophthalmologica	*Docum. Ophthal.*
Endeavour	*Endeavour*
Endocrinology	*Endocrinology*
Endokrinologie	*Endokrinologie*
Enzymologia	*Enzymologia*
Ergebnisse der Anatomie und Entwicklungsgeschichte	*Ergebn. Anat. EntwGesch.*
Ergebnisse der Physiologie	*Ergebn. Physiol.*
Experimental Medicine and Surgery	*Exp. Med. Surg.*
Fermentforschung	*Fermentforschung*
Fiziologicheskii Zhurnal (Moscow)	*Fiziol. Zhur.*
Folia haematologica	*Folia haemat., Lpz.*
Food Research	*Food Res.*
Growth	*Growth*
Guy's Hospital Reports	*Guy's Hosp. Rep.*
Haematologica	*Haematologica*
Handbuch der biochemischen Arbeitsmethoden	*Handb. biochem. ArbMeth.*
Handbuch der biologischen Arbeitsmethoden	*Hand. Biol. ArbMeth.*
Harvey Lectures	*Harvey Lect.*
Heart	*Heart*
Helvetica Physiologica et Pharmacologica Acta	*Helv. physiol. pharmacol. Acta*
Hoppe-Seylers Zeitschrift für physiologische Chemie	*Hoppe-Seyl. Z.*
Hormones and Vitamins	*Hormones and Vitamins*
Imperial Cancer Research Fund Scientific Reports	*Imp. Cancer Res. Fund Sci. Rep.*
Indian Journal of Medical Research	*Ind. J. med. Res.*

Periodical	Abbreviation
Jahresbericht Physiologie und experimentelle Pharmakologie	*Jber. Physiol. exp. Pharm.*
Japanese Journal of Medical Sciences	*Jap. J. med. Sci.*
Part 2. Biochemistry	*Biochem.*
Part 3. Biophysics	*Biophys.*
Johns Hopkins Hospital Bulletin	*Johns Hopk. Hosp. Bull.*
Johns Hopkins Hospital Reports	*Johns Hopk. Hosp. Rep.*
Journal de chimie physique (et Revue générale des colloïdes), électrochimie, thermochimie, radiochimie, mécanique chimique, stœchiométrie	*J. Chim. phys.*
Journal de l'anatomie et de la physiologie	*J. Anat., Paris*
Journal de pharmacie et de chimie	*J. Pharm. Chim., Paris*
Journal de physiologie et de pathologie générale	*J. Physiol. Path. gén.*
Journal de psychologie normale et pathologique	*J. Psychol. norm. path.*
Journal für Psychologie und Neurologie	*J. Psychol. Neurol., Lpz.*
Journal of Agricultural Research	*J. agric. Res.*
Journal of Anatomy	*J. Anat., Lond.*
Journal of Aviation Medicine	*J. Aviat. Med.*
Journal of Bacteriology	*J. Bact.*
Journal of Biochemistry	*J. Biochem, Tokyo*
Journal of Biological Chemistry	*J. biol. Chem.*
Journal of Biophysics	*J. Biophys., Tokyo*
Journal of Cancer	*J. Cancer, Lond.*
Journal of Cancer Research	*J. Cancer Res.*
Journal of Cellular and Comparative Physiology	*J. cell. comp. Physiol.*
Journal of Clinical Endocrinology	*J. clin. Endocrinol.*
Journal of Clinical Investigation	*J. clin. Invest.*
Journal of Clinical Research	*J. clin. Res.*
Journal of Comparative Neurology	*J. comp. Neurol.*
Journal of Dairy Research	*J. Dairy Res.*
Journal of Dental Research	*J. dent. Res.*
Journal of Endocrinology	*J. Endocrinol.*
Journal of Experimental Biology	*J. exp. Biol.*
Journal of Experimental Medicine	*J. exp. Med.*
Journal of Experimental Psychology	*J. exp. Psychol.*
Journal of Experimental Zoology	*J. exp. Zool.*
Journal of General Physiology	*J. gen. Physiol.*
Journal of Genetics	*J. Genet.*
Journal of Hygiene	*J. Hyg., Camb.*
Journal of Immunology	*J. Immunol.*
Journal of Industrial Hygiene	*J. industr. Hyg.*
Journal of Infectious Diseases	*J. infect. Dis.*
Journal of Laboratory and Clinical Medicine	*J. Lab. clin. Med.*
Journal of Medical Research	*J. med. Res.*
Journal of Metabolic Research	*J. metab. Res.*
Journal of Morphology	*J. Morph.*
Journal of Neurology and Psychopathology	*J. Neurol. Psychopath.*
Journal of Neuropathology and experimental Neurology	*J. Neuropath. exp. Neurol.*
Journal of Neurophysiology	*J. Neurophysiol.*
Journal of Nutrition	*J. Nutrit.*
Journal of Obstetrics and Gynaecology	*J. Obstet. Gynaec.*
Journal of Pathology and Bacteriology	*J. Path. Bact.*
Journal of Pharmacology and Experimental Therapeutics	*J. Pharmacol.*
Journal of Physiology	*J. Physiol.*
Journal of Scientific Instruments	*J. sci. Instrum.*
Journal of the American Chemical Society	*J. Amer. chem. Soc.*
Journal of the American Medical Association	*J. Amer. med. Ass.*
Journal of the American Pharmaceutical Association	*J. Amer. pharm. Ass.*
Journal of the Canadian Medical Association	*J. Canad. med. Ass.*
Journal of the Chemical Society	*J. chem. Soc.*
Journal of the Marine Biological Association	*J. Mar. biol. Ass. U.K.*
Journal of the National Cancer Institute	*J. Nat. Cancer Inst.*
Journal of the Optical Society of America	*J. opt. Soc. Amer.*
Journal of the Pharmaceutical Society of Japan	*J. pharm. Soc. Japan*
Journal of the Royal Microscopical Society	*J. R. micr. Soc.*
Journal of Urology	*J. Urol.*

Periodical	Abbreviation
Klinische Wochenschrift	*Klin. Wschr.*
Kolloidzeitschrift	*Kolloidzschr.*
Lancet	*Lancet*
Liebigs Annalen der Chemie	*Liebigs Ann.*
Luftfahrtmedizin	*Luftfahrtmed.*
Medical Journal of Australia	*Med. J. Aust.*
Medical Journal of South Africa	*Med. J. S. Afr.*
Medical Science, Abstracts and Reviews	*Med. Sci.*
Medico-Chirurgical Transactions	*Med.-chir. Trans.*
Mikrochemie	*Mikrochemie*
Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie	*Mitt. Grenzgeb. Med. Chir.*
Monatsschrift für Kinderheilkunde	*Mschr. Kinderheilk.*
Monitore zoologico italiano	*Monit. zool. ital.*
Morgagni	*Morgagni*
Morphologisches Jahrbuch	*Morph. Jb.*
Münchener medizinische Wochenschrift	*Münch. med. Wschr.*
Nature	*Nature, Lond.*
Naturwissenschaften	*Naturwissenschaften*
Nederlandsch Tijdschrift voor Geneeskunde	*Ned. Tijdschr. Geneesk.*
New Phytologist	*New Phytol.*
Nutrition Abstracts and Reviews	*Nutr. Abstr. Rev.*
Oppenheimer's Handbuch der Biochemie	*Handb. Biochem., Berl.*
Parasitology	*Parasitology*
Pflügers Archiv	*Pflüg. Arch. ges. Physiol.*
Philosophical Magazine	*Phil. Mag.*
Philosophical Transactions of the Royal Society	*Philos. Trans.*
Physikalische Zeitschrift	*Phys. Z.*
Physiological Abstracts	*Physiol. Abstr.*
Physiological Reviews	*Physiol. Rev.*
Phytopathologische Zeitschrift	*Phytopath. Z.*
Phytopathology	*Phytopathology*
Plant Physiology	*Plant Physiol.*
Policlinico	*Policlinico*
Presse médicale	*Pr. méd.*
Proceedings of the Mayo Clinic	*Proc. Mayo Clin.*
Proceedings of the National Academy of Sciences, Washington	*Proc. nat. Acad. Sci., Wash.*
Proceedings of the Nutrition Society	*Proc. Nutr. Soc.*
Proceedings of the Physical Society	*Proc. phys. Soc.*
Proceedings of the Physiological Society	*Proc. physiol. Soc.*
Proceedings of the Royal Academy of Sciences, Amsterdam	*Proc. Acad. Sci. Amst.*
Proceedings of the Royal Dublin Society	*Sci. Proc. R. Dublin Soc.*
Proceedings of the Royal Society	*Proc. Roy. Soc.*
Proceedings of the Royal Society of Canada	*Proc. Roy. Soc. Can.*
Proceedings of the Royal Society of Edinburgh	*Proc. Roy. Soc. Edinb.*
Proceedings of the Royal Society of Medicine	*Proc. R. Soc. Med.*
Proceedings of the Society of Experimental Biology and Medicine	*Proc. Soc. exp. Biol., N.Y.*
Proceedings of the Zoological Society of London	*Proc. zool. Soc. Lond.*
Publications of the South African Institute for Medical Research	*Publ. S. Afr. Inst. med. Res.*
Pubblicazioni della Stazione zoologica di Napoli	*Pubbl. Staz. zool. Napoli*
Quarterly Bulletin of the Health Organization, League of Nations	*Quart. Bull. Hlth Org. L.o.N.*
Quarterly Journal of Experimental Physiology	*Quart. J. exp. Physiol.*
Quarterly Journal of Medicine	*Quart. J. Med.*
Quarterly Journal of Microscopical Science	*Quart. J. micr. Sci.*
Quarterly Journal of Pharmacy and Allied Sciences	*Quart. J. Pharm.*

PERIODICAL	ABBREVIATION
Rendiconti della R. Accademia dei Lincei	*R.C. Accad. Lincei*
Reports of British Association	*Rep. Brit. Ass.*
Revue canadienne de biologie	*Rev. Canad. Biol.*
Revue française d'endocrinologie	*Rev. franç. Endocrin.*
Riforma Medica	*Rif. med.*
Rivista di biologia	*Riv. Biol.*
Science	*Science*
Science Abstracts	*Sci. Abstr.*
Sitzungsbericht der Akademie der Wissenschaften in Wien	*S.B. Akad. Wiss. Wien*
Skandinavisches Archiv für Physiologie	*Skand. Arch. Physiol.*
South African Journal of Medical Sciences	*S. Afr. J. med. Sci.*
South African Medical Journal	*S. Afr. med. J.*
South African Journal of Science	*S. Afr. J. Sci.*
Special Report Series Medical Research Council, London	*Spec. Rep. Ser. med. Res. Coun., Lond.*
Sperimentale. Archivio di biologia normale e patologica	*Sperimentale*
Stanford University Publications—University Series	*Stanford Univ. Publ.*
Biological Sciences	*biol. Sci.*
Medical Sciences	*med. Sci.*
Surgery, Gynecology and Obstetrics	*Surg. Gynec. Obstet.*
Tabulae biologicae	*Tabul. biol., Berl.*
Texas Reports on Biology and Medicine	*Texas Rep. Biol. Med.*
Tohoku Journal of Experimental Medicine	*Tohoku J. exp. Med.*
Transactions of the Faraday Society	*Trans. Faraday Soc.*
Transactions of the Optical Society	*Trans. opt. Soc. Lond.*
Transactions of the Royal Society of Canada	*Trans. Roy. Soc. Can.*
Transactions of the Royal Society, Edinburgh	*Trans. Roy. Soc. Edinb.*
Transactions of the Royal Society, South Africa	*Trans. Roy. Soc. S. Afr.*
Trabajos del Laboratorio de investigaciones biológicas de la Universidad de Madrid	*Trab. Lab. Invest. biol. Univ. Madr.*
Ugeskrift för Læger	*Ugeskr. Læg.*
United States Naval Medical Bulletin	*U.S. nav. med. Bull.*
University of California Publications	*Univ. Calif. Publ.*
Verhandeligen der (K) Akademie van Wetenschappen	*Verh. Akad. Wet., Amst.*
Verhandlungen der Gesellschaft Deutscher Naturforscher und Ärzte	*Verh. Ges. dtsch. Naturf. Ärzt.*
Verhandlungen des Vereins der Schweizer Physiologen	*Verh. Ver. Schweiz. Physiol.*
Verslagen van de gewone vergadering der wis- en natuurkundige afdeeling. Konink. Akademie van wetenschappen te Amsterdam	*Versl. gewone Vergad. Akad. Amst.*
War Medicine	*War Med.*
Wiener klinische Wochenschrift	*Wien. klin. Wschr.*
Wiener medizinische Wochenschrift	*Wien. med. Wschr.*
Zeitschrift für allgemeine Physiologie	*Z. allg. Physiol.*
Zeitschrift für Augenheilkunde	*Z. Augenheilk.*
Zeitschrift für Biologie	*Z. Biol.*
Zeitschrift für die gesamte Anatomie	*Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* 2. *Z. KonstLehre.* 3. *Ergebn. Anat. EntwGesch.*
Zeitschrift für die gesamte experimentelle Medizin	*Z. ges. exp. Med.*
Zeitschrift für die gesamte Neurologie und Psychiatrie	*Z. ges. Neurol. Psychiat.*
Zeitschrift für die medizinische Wissenschaften	*Z. med. Wiss.*
Zeitschrift für experimentelle Pathologie und Therapie	*Z. exp. Path. Ther.*
Zeitschrift für Hygiene und Infektionskrankheiten	*Z. Hyg. InfektKr.*
Zeitschrift für Immunitätsforschung	*Z. ImmunForsch.*
Zeitschrift für Kinderheilkunde	*Z. Kinderheilk.*
Zeitschrift für klinische Medizin	*Z. klin. Med.*
Zeitschrift für Kreislaufforschung	*Z. KreislForsch.*
Zeitschrift für mikroscopisch-anatomische Forschung	*Z. mikr.-anat. Forsch.*
Zeitschrift für physikalische Chemie	*Z. phys. Chem.*

PERIODICAL	ABBREVIATION
Zeitschrift für physiologische Chemie	*Hoppe-Seyl. Z.*
Zeitschrift für Psychologie und Physiologie der Sinnesorgane	*Z. Psychol. Physiol. Sinnesorg.*
Zeitschrift für Sinnesphysiologie	*Z. Sinnesphysiol.*
Zeitschrift für vergleichende Physiologie	*Z. vergl. Physiol.*
Zeitschrift für Vitaminforschung	*Z. Vitaminforsch.*
Zeitschrift für wissenschaftliche Zoologie	*Z. wiss. Zool.*
Zeitschrift für Zellforschung und mikroskopische Anatomie	*Z. Zellforsch.*
Zentralblatt für allgemeine Pathologie und pathologische Anatomie	*Zbl. allg. Path. path. Anat.*
Zentralblatt für Bakteriologie, Parasitenkunde u. Infektionskrankheiten (Abteilung 1, Originale or Referate)	*Zbl. Bakt.* (1. Abt. Orig. or Ref.)
Zentralblatt für Bakteriologie, Parasitenkunde u. Infektionskrankheiten (Abteilung 2)	*Zbl. Bakt.* (2. Abt.)
Zentralblatt für Biochemie und Biophysik	*Zbl. Biochem. Biophys.*
Zentralblatt für die gesamte Gynäkologie und Geburtshilfe	*Zbl. ges. Gynäk. Geburtsh.*
Zentralblatt für die medizinische Wissenschaften	*Zbl. med. Wiss.*
Zentralblatt für gesamte Physiologie und Pathologie des Stoffwechsels	*Zbl. ges. Physiol. Path. Stoffw.*
Zentralblatt für Physiologie	*Zbl. Physiol.*

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THE EFFECT OF MAGNESIUM AND CALCIUM ON THE PHYSIOLOGICAL PROPERTIES OF CERTAIN PURINE DERIVATIVES

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We have described previously some of the pharmacological actions of adenosine triphosphate and related compounds. The purpose of this work is to show how and to what degree Mg and Ca affect the circulatory reactions to these purine derivatives. It has already been reported (Bielschowsky & Green, 1944) that the Mg salt of adenosine triphosphate is more toxic than the Na salt and that free Mg^{++}* is capable of potentiating the toxic action of this and other purine derivatives (Green & Stoner, 1944). Consequently, it was important to determine the effect of Mg on other properties of these compounds. To this end, the circulatory effects of the Na and Mg salts of these substances were compared, and the action of free Mg^{++} on these effects observed. Since Ca antagonizes some of the pharmacological effects of Mg, its influence on these compounds was also studied. Drury & Szënt-Györgyi (1929) have already observed the effect of free Ca^{++} on the response of the heart to adenylic acid and adenosine and found that there was no alteration with doses up to 5 mg. The only substances found to modify the action of these two compounds were Ba and phosphotungstic acid, the former decreasing and the latter intensifying their action on the heart.

Methods

The investigation was carried out on a series of forty-nine cats, three rabbits and one dog, anaesthetized either with nembutal or chloralose. The results obtained, with one exception which will be mentioned later, were identical with both anaesthetics. The blood pressure was recorded from the carotid artery with a mercury manometer. The intravenous injections were given into the external jugular vein or a radicle of the portal vein and washed in with physiological salt solution from a burette. In some experiments the contractions of the left ventricle were recorded with a Cushny myograph and the contractions of the right auricle by a thread attached to its apex. Intestinal volume changes were recorded with an oncometer connected to a tambour recorder and, in some experiments, a blood-pressure compensator (Bayliss, 1908) was attached to a cannula in the iliac artery. At the beginning of the majority of the experiments in which free Mg^{++} was used, both ureters were ligated to stabilize the level of the blood Mg (Hoff, Smith & Winkler, 1939).

* This term signifies that Mg^{++} was injected separately.

The purine derivatives used were adenosine (A), the Na and Mg salts of muscle adenylic acid (A5 MP), yeast adenylic acid (A3 MP), adenosine diphosphate (ADP), adenosine triphosphate (ATP) and inosine triphosphate (ITP). Adenosine was obtained from British Drug Houses Ltd., yeast adenylic acid was prepared from yeast nucleic acid, and the remainder were prepared from the Ba salt of ATP. The purity of these compounds was checked by analysis of their N, pentose, total and 7′ P contents. Only those preparations were used which, on the basis of these analyses, were found to be at least 98% pure.

Magnesium ($MgSO_4.7H_2O$) was injected either intravenously as a 0·154 M. or 0·077 M. solution or intraperitoneally as a 1·02 M. solution. Neither the nature of the anion (e.g. Mg lactate) nor the route of administration affected the response. Calcium ($CaCl_2$) was given intravenously in isotonic solutions (0·133 M.).

Results

*The effect of 'combined' magnesium**

Using the Ca salts of ATP and A5 MP, Gillespie (1934) found the vaso-depressor activity of the phosphorylated derivatives in the cat and rabbit to be A > A5 MP > ATP. Using the Na and Mg salts we found this order was reversed. There seemed no doubt that ATP was the most active of the three.

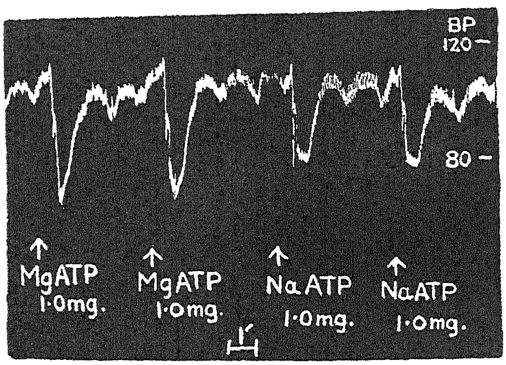

Fig. 1. Record of arterial blood pressure (BP) from a cat (1·75 kg. body wt.) under nembutal anaesthesia. Comparison of the depressor activities of NaATP and MgATP.

Of the two salts of ATP tested, the Na possessed about two-thirds of the depressor activity of the Mg salt (Fig. 1). The transient primary rise in blood pressure described by Gillespie (1934) was rarely seen in the cat after the injection of either the Na or Mg salt of ATP.

ATP is known to produce a fall in blood pressure by two mechanisms—primarily by depressing the action of the heart and secondarily by causing peripheral vaso-dilatation. McDowall (1944) has shown that, in the cat, its action on the heart is further divisible into a central depressant action through the vagus and a direct depressant action on cardiac muscle. We have confirmed this observation (Fig. 2); but have found that, of all the compounds tested, ATP is the only one which acts through the vagus. Moreover, vagal depression

* This term signifies that Mg salts of the nucleotides were injected.

though seen in the cat (it may possibly also occur in man) plays no part in the depressor activity of ATP in the rabbit.

The Mg salt of ATP was found to have a somewhat greater action on the heart than the Na salt (Fig. 3). This effect was only clearly seen when the

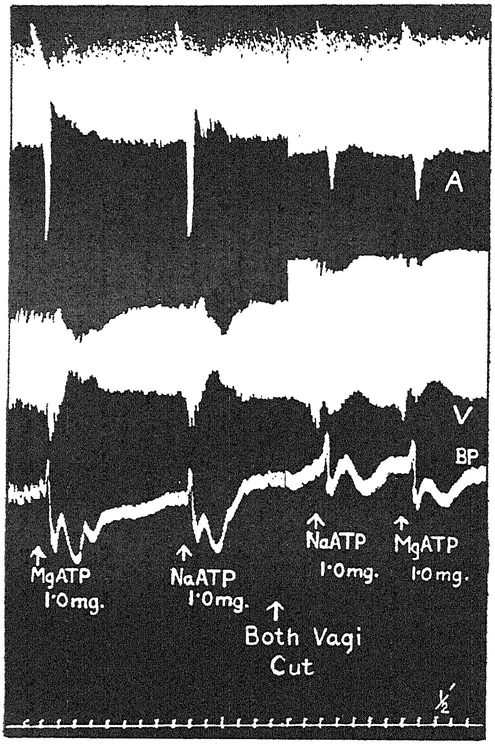

Fig. 2.

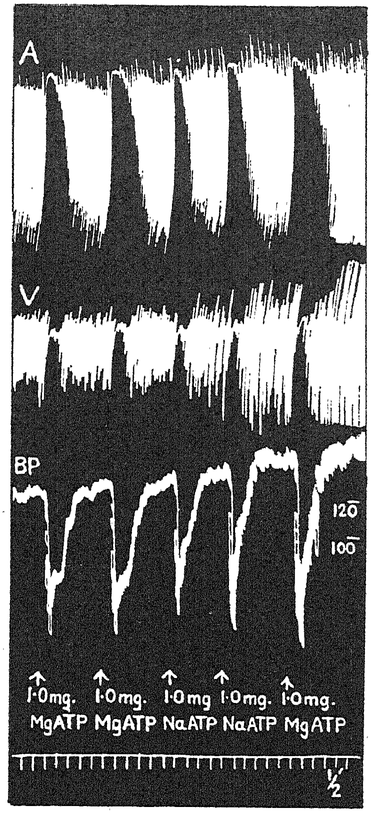

Fig. 3.

Fig. 2. Record of arterial blood pressure (BP) and cardiac contractions from a cat (3·9 kg. body wt.) under chloralose anaesthesia. Effect of NaATP and MgATP before and after section of the vagi. A = right auricular tracing; V = left ventricular tracing.

Fig. 3. Record of arterial blood pressure (BP) and cardiac contractions from a cat (2·6 kg. body wt.) under chloralose anaesthesia. Comparison of the cardiac actions of MgATP and NaATP. A = right auricular tracing; V = left ventricular tracing.

vagi were intact (Figs. 2, 3). Observations on intestinal volume changes with the Bayliss compensator in the circuit indicate that it may also have a greater vaso-dilator action than the Na salt.

After deamination of ATP its depressor activity is reduced twenty-fold, and the slight residual activity was found to be the same for both the Na and Mg

salts of ITP. This result is in contrast to the high toxicity of MgITP as compared with that of NaITP (Bielschowsky & Green, 1944).

After the removal of one phosphate group from ATP, the resultant diphosphate (ADP) had, at the most, one-third of the depressor activity of the original ATP. It depressed the blood pressure mainly through its action on the heart by slowing the ventricular beat and diminishing the amplitude of contraction (Fig. 4). The Mg salt was slightly more active than the Na salt; for instance, in the experiment of Fig. 4, the effect of the Mg salt on the

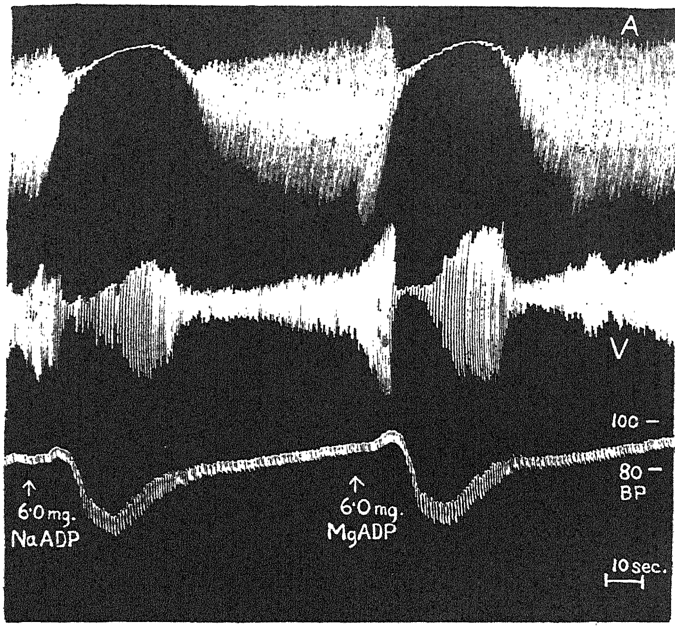

Fig. 4. Record of arterial blood pressure (BP) and cardiac contractions from a cat (2·35 kg. body wt.) under chloralose anaesthesia. Comparison of NaADP and MgADP. *A* = right auricular tracing; *V* = left ventricular tracing.

ventricle was stronger than that of the Na salt. When ADP was given in doses under 4·0 mg. it still inhibited the ventricular beat but had little or no effect on the auricle. In this respect ADP differs from A5 MP and A.

The removal of both phosphate groups only leads to a slight further reduction in depressor activity and, with the exception of those experiments in which A5 MP salts were tested on cats in nembutal anaesthesia, the Mg salt again had a stronger depressor effect than the Na salt. The difference, however, was only very slight (for A3 MP see Fig. 5 and for A5 MP see Fig. 10). Under nembutal anaesthesia the Mg salt of A5 MP was slightly less depressant than

the Na salt, probably because the immediate fall was shortened by a secondary rise in blood pressure accompanied by cardiac acceleration (Fig. 5). This secondary rise has not been analysed.

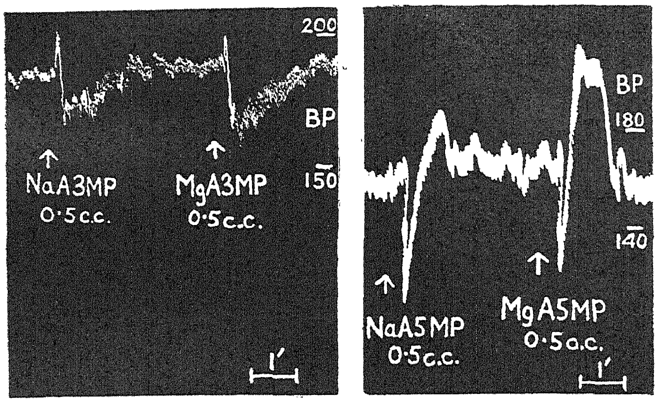

Fig. 5. Records of arterial blood pressure (BP) from two cats (1·8 and 2·0 kg. body wt.) under nembutal anaesthesia. Comparison of the effect of the Na and Mg salts of A5 MP and A3 MP. Dose in each case 0·5 c.c. of an equimolecular solution containing 3·47 mg. AMP per c.c.

The effect of free magnesium ion

We have confirmed that Mg^{++} when injected parenterally is depressant to both the central nervous system (Meltzer & Auer, 1905) and to the heart (Mathews & Jackson, 1907) and, as Hoff *et al.* (1939) have shown, it is a powerful vaso-dilator. On the basis of these investigations, and the finding that the Mg^{++}-injected animal was much more sensitive to the toxic effects of ATP and its derivatives (Green & Stoner, 1944), it was to be expected that free Mg^{++} would increase still further the depressor action of intravenous ATP. Actually the reverse effect was found, for free Mg^{++} reduced the depressor activity of intrajugular ATP (Fig. 6). The solution of this apparently paradoxical finding was the main stimulus to this investigation. The Mg salt was more susceptible to this action of Mg^{++} than the Na salt. The intravenous doses of Mg^{++} required are, from the pharmacological aspect, relatively small, a reduction of the depressor response being produced by as little as 40 mg. $MgSO_4$ (anhydrous)/kg. body wt. Of the other purine derivatives studied both A5 MP (Fig. 13) and A3 MP (Fig. 11) showed a reduction in their depressor activities after $MgSO_4$. The reduction, however, was less than that observed with ATP, and larger amounts of $MgSO_4$ (160 mg./kg. body wt.) were required to produce an effect. The depressor activities of ADP (Fig. 8) and A (Fig. 6) were scarcely, if at all, affected by Mg^{++}, but a small decrease in the cardiac response to these substances was consistently seen (Figs. 7, 8). The very slight depressor activity of ITP (i.e. deaminated ATP) was unaffected by Mg^{++}.

The reduction of the depressor activities of ATP and A5 MP by Mg^{++} may be explained to a great extent by the fact that Mg^{++} reduced the cardio-inhibitory effect of these substances. This is shown for ATP in Fig. 7. In addition, since the depressor response to A3 MP, which does not affect the heart, is also reduced, $MgSO_4$ would also seem to reduce the vaso-dilator activities of these purine derivatives.

The possibility exists that this effect of Mg^{++} may be specific for purine compounds containing an amino group, for the vaso-depressor properties of a vaso-dilator such as histamine (Fig. 6) and a cardiac depressant such as acetylcholine were unaffected.

In explaining the fact that $MgSO_4$ decreased the depressor response of ATP to a much greater extent than that of the other adenosine compounds it must

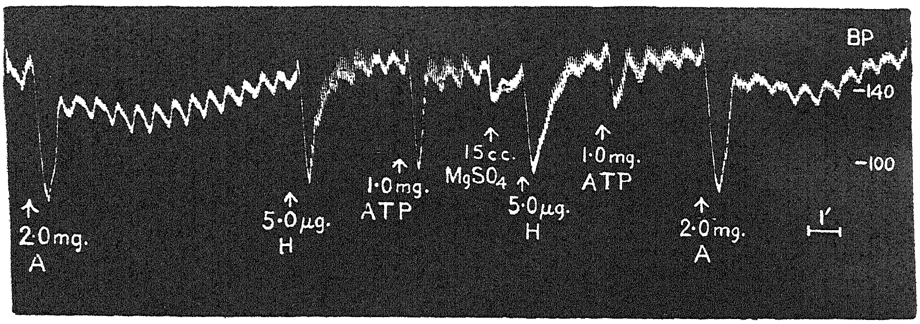

Fig. 6. Record of arterial blood pressure (BP) from a cat (2·5 kg. body wt.) under nembutal anaesthesia. Effect of an intravenous injection of $MgSO_4$ (0·077 M.) on the depressor activity of A, ATP and histamine (H).

be remembered that ATP acts in the cat largely through the vagus. It is known from the work of Meltzer & Auer (1906) and MacNider & Mathews (1907) that Mg^{++} reduces the sensitivity of the vagal nuclei with their afferent and efferent connexions, and we have found that this is true of the doses of Mg^{++} used in these experiments. This would seem to explain the ease with which in the cat the depressor response to ATP is reduced after injecting Mg^{++}. Evidence in favour of this view was found in the rabbit, in which ATP does not act through the vagus and in which larger amounts of Mg^{++} were required than in the cat to produce a similar effect. However, the whole effect cannot be explained by depression of vagal activity, for the response in the cat with both vagi cut is also reduced (Fig. 7, II), and, as already stated, the depressor response to ATP in the rabbit and to other adenosine compounds having no vagal action is also affected.

It remains therefore to explain those effects of Mg^{++} not accounted for by its action on the vagal mechanism. One possibility is that since Mg^{++} is a

powerful vaso-dilator it increases permeability so that these compounds leave the circulation more rapidly and thus have a more transient depressor action.

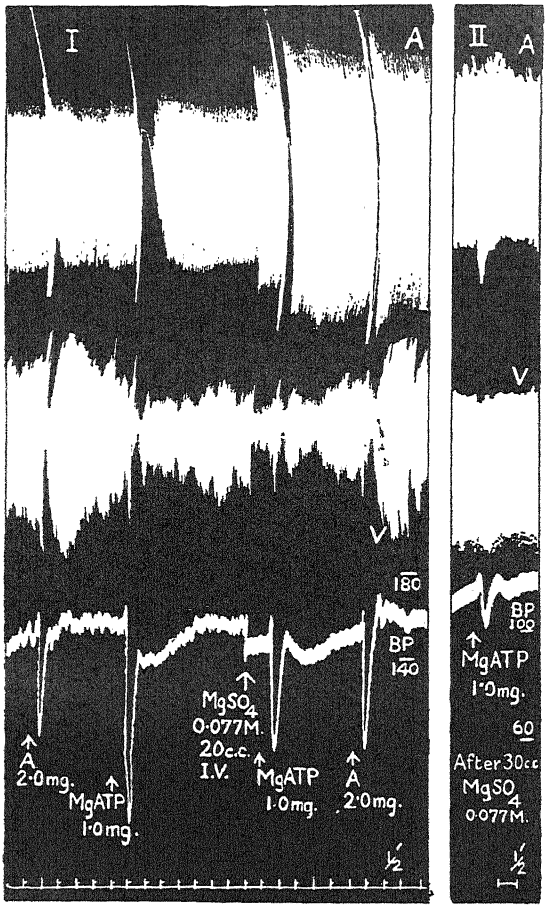

Fig. 7.

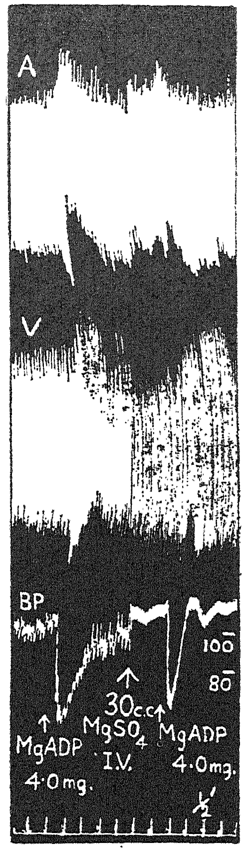

Fig. 8.

Fig. 7. Records of arterial blood pressure (BP) and cardiac contractions from cats under chloralose anaesthesia. *A* = right auricular tracing; *V* = left ventricular tracing. I. Cat, 3·38 kg. body wt. Shows effect of an intravenous injection of $MgSO_4$ on the action of A and ATP on the heart and blood pressure. II. Cat, 3·9 kg. body wt. Same cat as Fig. 2, with which it should be compared. Shows that after section of the vagi there is still a reduction in the cardiac and depressor effects of ATP following intravenous $MgSO_4$ (30 c.c. 0·077 M.).

Fig. 8. Record of arterial blood pressure (BP) and cardiac contractions from a cat (2·7 kg. body wt.) under chloralose anaesthesia. Effect of an intravenous injection of $MgSO_4$ (0·077 M.) on the response to MgADP. *A* = right auricular tracing; *V* = left ventricular tracing.

On this hypothesis it is difficult to understand why Mg^{++} should reduce the effects of ATP and some related compounds on cardiac muscle.

An alternative explanation arises from the concept that some part of the circulatory effects of all these compounds containing adenosine is due to a common breakdown product (e.g. NH_3). Inhibition by Mg^{++} of the deaminating enzymes concerned might then explain our results. It has been shown that Mg^{++} does in fact inhibit the deamination *in vitro* of ATP, A5 MP and A (Stoner & Green, 1945*b*), and the findings following the injection of two of these substances into the portal circulation are compatible with its having a similar action *in vivo*. When ATP and A were injected intraportally into the cat their effect on the blood pressure was greatly diminished. This might be due to their distribution in the vascular system or might possibly result

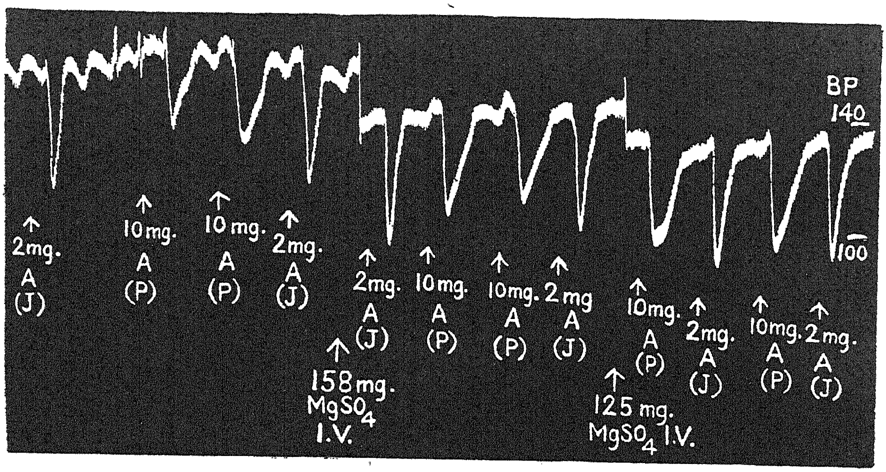

Fig. 9. Record of arterial blood pressure (BP) from a cat (1·95 kg. body wt.) under nembutal anaesthesia. Response to intraportal injection of A after intravenous injection of $MgSO_4$ (0·154 M.). P = injected into the portal vein. J = injected into the external jugular vein.

from their deamination in the liver. After the administration of free Mg^{++}, adenosine injected intraportally had a greater effect on the blood pressure (Fig. 9). The response to intraportal ATP remained the same before and after the injection of free Mg^{++}. Since the blood-pressure response to intrajugular ATP was reduced by half by the amount of Mg^{++} used it would seem that, following Mg^{++} administration, twice as much ATP escaped from the liver intact. More than one interpretation of these results is possible, but of these, that which ascribes the action of Mg^{++} to inhibition of the deamination of these compounds is favoured.

The effect of calcium

Free Ca^{++} was found to increase the depressor activity, after intravenous injection, of all the purine derivatives studied except A3 MP. The order of

sensitivity was the reverse of that to Mg^{++}, adenosine being the most easily affected. The intravenous dose of Ca^{++} required to produce the effect was 30–40 mg. $CaCl_2$/kg. body wt.

The mode of action of Ca^{++} appears to be that of increasing the sensitivity of the heart to the depressor action of these compounds (Fig. 10). The

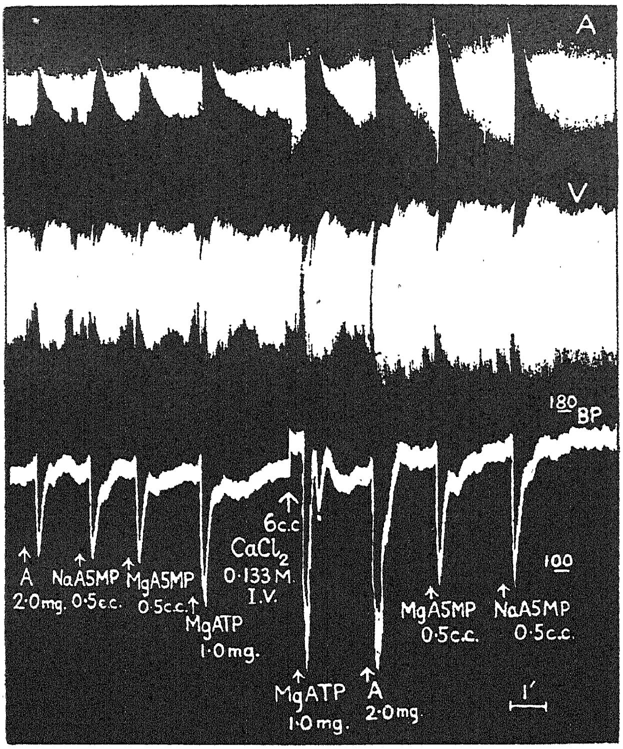

Fig. 10. Record of arterial blood pressure (BP) and cardiac contractions from a cat (3·25 kg. body wt.) under chloralose anaesthesia. Shows the effect of intravenous injection of free Ca^{++} ($CaCl_2$) on the actions of ATP, A5 MP and A. A5 MP salts given in equimolecular solutions containing 3·47 mg. AMP per c.c. *A* = right auricular tracing; *V* = left ventricular tracing.

sensitivity of both auricle and ventricle is increased, and the reaction is unaffected by cutting the vagi. This increased sensitivity of the auricle was well shown with ADP, for doses which had no action on the auricle became depressant after the injection of Ca^{++}. Unlike the opposite effect with Mg^{++}, the Ca^{++} effect was not lost after deamination of ATP. ITP has a slight cardio-depressor action (Drury, 1936) which is increased by Ca^{++}; on the other hand, the effect of A3 MP, which does not depress the cardiac mechanism, was unaltered by Ca^{++} (Fig. 11).

Ca-Mg antagonism was first demonstrated in Mammalia by Meltzer & Auer (1908), and it is now well known that the animal in Mg anaesthesia can be restored to full consciousness by Ca^{++}. Ca^{++} antagonizes the depressor action of Mg^{++} on both the nervous and vascular systems. It was of interest

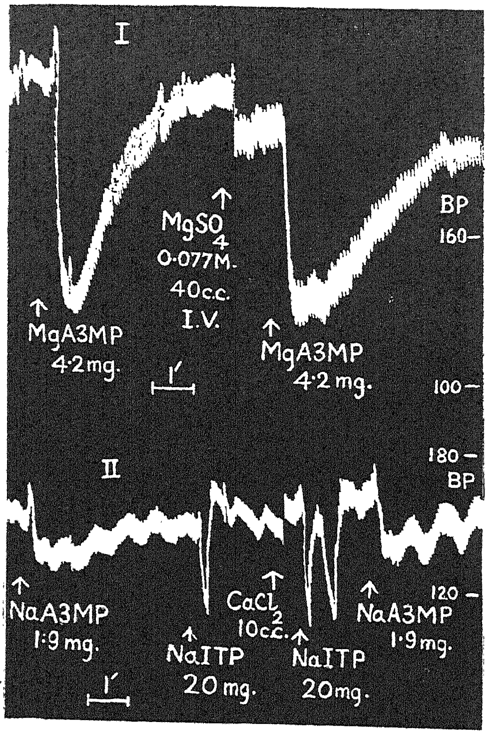

Fig. 11.

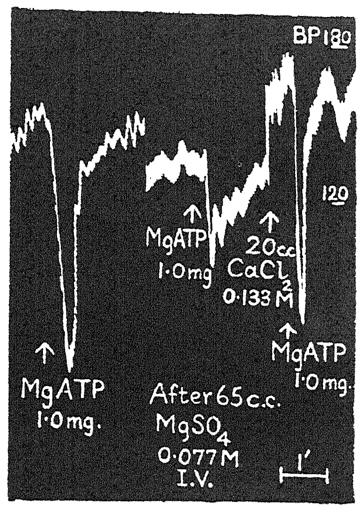

Fig. 12.

Fig. 11. Records of arterial blood pressure (BP) from two cats under nembutal anaesthesia. I. Effect of intravenous injection of $MgSO_4$ on the depressor activity of A3 MP. Cat 2·9 kg. body wt. II. Effect of intravenous injection of $CaCl_2$ (0·133 M.) on the depressor activity of ITP and A3 MP. Cat 2·85 kg. body wt.

Fig. 12. Record of arterial blood pressure (BP) from a cat (3·6 kg. body wt.) under nembutal anaesthesia. Shows the reduction in the MgATP response produced by free Mg^{++} and the reversal of this effect by free Ca^{++}.

therefore to discover whether Ca^{++} would reverse the effect of Mg^{++} on purine derivatives. This proved to be so (Figs. 12, 13). As might be expected the ease of reversal was inversely proportional to the ease with which the depressor response was reduced by Mg^{++}. The dose of Ca^{++} required to reverse the Mg^{++} effect on the response to A5 MP was accordingly less than that required to reverse the effect of the same amount of Mg^{++} on the response to ATP.

This is shown in Fig. 13. The depression by Mg^{++} of the response to ATP in the cat with cut vagi could also be reversed by Ca^{++}. The reversing dose of Ca^{++} obviously depended on how much Mg^{++} the animal had received (cf. Figs. 12, 13). The Mg effect was, however, always dominant and has been produced in a cat with a serum Ca level of 28 mg./100 c.c.

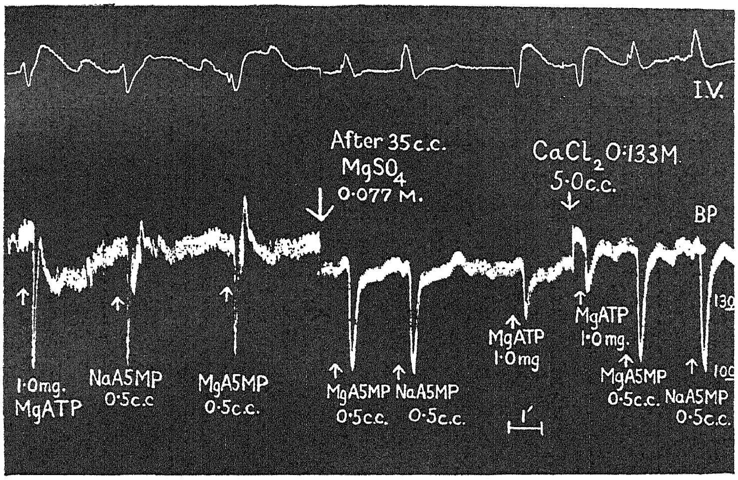

Fig. 13. Recording of arterial blood pressure (BP) and intestinal volume (I.V.) from a cat (2·1 kg. body wt.) under nembutal anaesthesia. Shows the effect of an intravenous injection of $MgSO_4$ on the blood pressure and intestinal volume responses to ATP and A5 MP and their restoration (partial in the case of ATP) by free Ca^{++}.

Discussion

It has been seen that the action of certain purine derivatives on the cardiovascular system is increased when Mg is the cation, the increase being especially noticeable in the case of ATP. This is true, not only of the animals investigated here, but also of man (Stoner & Green, 1945*a*). The toxic effect of some of these compounds on the whole animal is also increased when Mg is the cation (Bielschowsky & Green, 1944). It would seem then that the introduction of Mg into the molecule enhances its biological action as a whole. The degree of dissociation of the Mg salts of the nucleotides in biological fluids is not known. It is, however, established (Tabor & Hastings, 1943) that acid Mg phosphates are only partly dissociated and that Mg occurs in blood in the free and bound forms (Dine & Lavietes, 1942). It is therefore probable that the Mg salts of the nucleotides are also only partly dissociated in biological fluids. Since it is probable that ATP exists in muscle as the Mg salt (Szënt-Györgyi, 1943; Bielschowsky & Green, 1944), the behaviour of the Mg salt may reflect its physiological action more closely.

The reduction of the depressor response by free Mg^{++} was unexpected, as it was known to increase the toxicity of these compounds. In the case of ATP

it is probable that in one species (cat) the effect is partly due to the depressor action of Mg^{++} on the central vagal mechanism, but, as already emphasized, this cannot be the explanation for its action in the rabbit and on compounds other than ATP. The additional hypotheses already suggested are that Mg^{++} increases the permeability of the capillaries or that it delays the breakdown of the purine compounds. Indeed, both mechanisms may be concerned. A rather similar phenomenon described by Winter & Barbour (1927) and Barbour & Winter (1927, 1929)—the potentiation of certain antipyretics by Mg—was interpreted by the authors as due to Mg^{++} increasing cell permeability. One great difficulty in this hypothesis is that there is no work, to our knowledge, specifically demonstrating such an action of Mg^{++} on vessel permeability. On the whole we favour the second hypothesis.

Inhibition of some enzyme concerned in the breakdown of ATP has already been suggested as an explanation of the effect of free Mg^{++} on the toxicity of these compounds (Green & Stoner, 1944). The arguments used there are not entirely applicable here, for depressor activity and toxicity appear to be predominantly functions of different parts of the nucleotide molecule. Given a basal purine-ribose linkage, toxicity would seem to be largely a function of the phosphate content of the molecule, whereas depressor activity is almost entirely dependent on the adenine amino group, albeit influenced, as shown in the present work, by the number of attached phosphate groups. Although it has been denied by Parnas & Ostern (1931) that deamination is responsible for the pharmacological actions of adenyl compounds, it is difficult to avoid the concept that their circulatory effects are in some degree associated with this process. If the latter view is correct we are more concerned here with the effect of Mg^{++} on deamination than on dephosphorylation except in so far as in some tissues (liver, kidney and ventricular muscle), under certain conditions, dephosphorylation precedes deamination (Conway & Cooke, 1939). The effect of Mg^{++} on deamination has not received much attention. Conway & Cooke (1939) and Brady (1942) found Mg to be without action on the rate of deamination of A and A5 MP, but the earlier work of Ostern & Mann (1933), using A5 MP and ATP, showed it to be a powerful inhibitor of these reactions in skeletal and cardiac muscle *brei*. We have confirmed that Mg^{++} has an inhibitory action on deamination *in vitro* (Stoner & Green, 1945*b*). Our results, following intraportal injections, suggest that it may also act similarly *in vivo*, though it is admittedly difficult to reconcile these results with the view that this is the mode of action of Mg^{++} in diminishing the circulatory effects of these compounds. The apparent discrepancies in this hypothesis may, however, prove later not to be real.

From our findings it is difficult to decide whether the opposite effect of Ca^{++} is general or a purely local one on the heart. As the depressor action of A3 MP, which has no direct action on the heart, is unaffected by Ca^{++}, the

latter view seems probable. We have no evidence as to whether the action of Ca^{++} is specific for these depressor substances studied or whether it is due to an increased sensitivity to any depressant stimulus. There is no good evidence that Ca^{++} even in excess decreases capillary permeability, nor does it appear to influence deamination (Stoner & Green, 1945*b*). That Ca-Mg antagonism occurs in the case of ATP is understandable, for Ca will antagonize the central vagal anaesthetic action of Mg; for the other compounds it is doubtful whether it has a specific antagonism.

The effects of both Mg^{++} and Ca^{++} on the physiological properties of these purine derivatives lack a complete explanation. This investigation arose out of a study of traumatic shock, and the relationship of these findings to the general problem is discussed elsewhere (Green & Stoner, 1944).

Summary

1. The effects of Mg and Ca on the cardiovascular reactions following the intravenous injection of the purine compounds, adenosine triphosphate, inosine triphosphate, adenosine diphosphate, muscle and yeast adenylic acids and adenosine, were studied, mainly on the cat.

2. The presence of 'combined' Mg in the molecule increases the activity of some of these compounds, especially adenosine triphosphate.

3. In contrast, the parenteral injection of free Mg^{++} usually decreases the intensity of some or all of their immediate cardiovascular effects. This effect is reversed by Ca^{++}.

4. When adenosine and adenosine triphosphate are injected into a radicle of the portal vein their effects are greatly reduced. Under these conditions free Mg^{++} appears to diminish the rate of deamination of these compounds in the liver.

5. The effect of Mg^{++} on the depressor action of adenosine triphosphate in the cat is in large part explained by its depressant action on the central vagal mechanism through which adenosine triphosphate largely produces its vaso-depressor effect. The results in the rabbit, and with other compounds in the cat, cannot be explained easily in this way, and to explain these results the hypothesis is advanced that Mg^{++} acts by diminishing the rate of deamination of these compounds.

6. The parenteral injection of free Ca^{++} increases the vaso-depressor response to these compounds with the exception of yeast adenylic acid. This may be due to an increase in the sensitivity of the heart to depressor substances in general.

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A COMPARISON OF THE ACTION OF VITAMIN D ON THE TEETH OF RACHITIC RATS WITH THAT OF ADDITIONAL CALCIUM OR PHOSPHORUS ADDED TO RACHITOGENIC DIETS

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In a previous paper (Irving, 1944*a*) it was shown that vitamin D caused histological changes in the teeth of rats which differed according as to whether the rats had been on a diet with a high or low Ca : P ratio. No such differences could be observed in the bones, since low-ratio diets do not cause widening of the epiphyses. It appeared to be of interest to compare the changes in the teeth of rachitic rats caused by vitamin D with those induced by altering the Ca : P ratio of the diet to one more favourable for calcification. Other workers (Karelitz & Shohl, 1927; Kramer, Shear & Siegel, 1931; Key & Morgan, 1932; Nicolaysen & Jansen, 1939; Coward & Kassner, 1940) have reported the changes in the bones, and in the blood chemistry, when P is administered to rachitic rats on high Ca : P ratio diets, but the changes in the teeth when this is done, and the effects produced by improving the ratio of a low Ca : P ratio diet, have not been investigated. The following experiments were therefore undertaken.

Methods

Animals. As before, young albino rats of the Wistar Institute strain were employed in litters of from six to eight. The conditions under which they were housed were the same as previously reported. When the rats weighed between 50 and 60 g. they were transferred to one of the experimental diets.

Diets of the experimental animals. Six experimental diets were employed; they were based on the Steenbock & Black rachitogenic diet no. 2965 (1925), the Ca and P contents being altered by the addition of various amounts of $CaCO_3$ (B.P.) and Na_2HPO_4, anhyd. (Merck *puriss.*). Table 1 gives the composition of the diets, the figures being checked by analysis.* For simplicity, the diets are referred to in the text by the number of the Ca : P ratio. The first three were similar to those previously employed. The composition of diet 5·6 varied somewhat from one batch to another. The figures given for

* The P content of the basal diet, without $CaCO_3$ or Na_2HPO_4, was erroneously given as 0·0206% in the previous paper (Irving, 1944*a*). This should have been 0·206%.

Table 1

Ca : P ratio and no. of diet	Ca %	P %	Description
1·9	1·43	0·77	'Normal' Ca : P ratio
5·6	1·39	0·25	High Ca : P ratio
0·26	0·098	0·37	Low Ca : P ratio
3·7	1·49	0·40	High Ca : P ratio, intermediate between 1·9 and 5·6
3·0	1·48	0·49	
2·5	1·32	0·52	

this diet are the averages of analyses of several samples. The diets were given slightly moistened with water. When vitamin D was given, it was in the form of irradiated ergosterol in oil (the international standard preparation) and was administered in one dose by mouth using a micrometer syringe.

Treatment of animals. In all cases the animals were maintained on diet 5·6 or 0·26 for 28 days. The following procedures were then adopted:

(*a*) Two animals of a litter were kept on the original experimental diet for a further 10 days; the rest of the litter was placed on diet 1·9 for 10 days. All the animals were then killed and examined.

(*b*) One animal was killed. The rest of the litter were transferred to diet 1·9 and were killed and examined at daily intervals.

(*c*) One animal was killed as a control. Half the rest of the litter were transferred to diet 1·9, the other half being given 30 i.u. of vitamin D. The animals were then killed after 1, 2, 4 or 6 days.

(*d*) Animals were transferred to diet 1·9 and killed at 6-hourly intervals up to 30 hr.

(*e*) Animals were transferred to diets 3·7, 3·0 or 2·5 and were killed at daily intervals.

Weighing of the rats. The animals were weighed weekly while on the first experimental diet, and three times, at 3-day intervals, after transference to the second experimental diet in procedures (*a*), (*b*), (*c*) and (*e*).

Examination of the rats. Longitudinal sections of the upper incisors were examined in the way previously described. The same method of measuring the time of action of vitamin D or of the new diet was used, but NaF injections were not given. The line test was applied to the lower ends of the radius and ulna (Coward, 1938). Some of the bones were also studied histologically. The lower ends of the radius and ulna were cut longitudinally on the freezing microtome without preliminary decalcification, treated with silver nitrate and in some cases also stained with haematoxylin.

Results

Animals transferred from diet 5·6 *to diet* 1·9

All the animals gained weight while on diet 5·6. On changing to diet 1·9, weight was still gained in all rats, save those of one litter on procedure (*c*). These animals all lost weight after the change of diet, but were making it up

as the experiment proceeded. Careful examination showed that this weight loss made no difference to the results, which were the same as those found in animals gaining weight. Karelitz & Shohl (1927) found that the addition of P to a high Ca : P ratio rachitogenic diet rendered the rats hyperexcitable and liable to attacks of tetany. No such effect was seen in the present experiments.

One litter of rats was treated with procedure (*a*). The control animals had marked rickets, while those transferred to diet 1·9 had, after 10 days, stages of healing in the epiphyses varying from 3 to 6 (Coward, 1938). The teeth of the control animals had all the signs of poor calcification with wide predentin and vascular inclusions. The teeth of the animals transferred to diet 1·9 showed a broad stripe of reactive new dentin in the more distal part, staining deeply with haematoxylin (Pl. 1, fig. 3), and, more proximally, a mass of interglobular dentin. The predentin between the reactive area and the old dentin tended to fill in with interglobular dentin. Measurements of the reactive dentin showed that the influence of diet 1·9 had begun within a day of changing to this diet.

Three litters of rats were treated with procedure (*b*). The line test became positive in all cases after 2 days on diet 1·9. The type of response seen in the bones during the early stages was in many respects, however, not like the changes after giving vitamin D (Pl. 3, fig. 12). It appeared to be a kind of disorderly healing (Pl. 3, fig. 13). Sometimes a separate line would be seen, as in the early stages of healing after vitamin D, but, more often, abnormal downgrowths of calcified material in the metaphysis joined the line to the diaphyseal bone. In several cases, the line was absent or not complete, and the downgrowths were a very notable feature. It was often impossible to grade the healing into one of the usual stages.

The pattern of change in the tooth was the same in all cases. After 1 day on diet 1·9, a narrow deeply staining stripe of new reactive dentin was seen distally on both the lingual and labial sides. This stripe widened on the lingual side during successive days and was often striated, so that the stripes could be counted. When this was so, one stripe corresponded to each day's action. On the labial side, the stripe again widened with successive days' action and also extended proximally. At about the time that it first appeared, a coarse sprinkling of calcospherites began throughout the intermediate and proximal predentin (Pl. 1, fig. 4). These built up in masses from near the already calcified dentin and spread over to the odontoblast side of the predentin, where they gradually fused to produce a stripe backed by much interglobular dentin and laid down at normal predentin width from the odontoblasts (Pl. 1, fig. 5). This stripe joined with that formed more distally.

In order to compare this reaction with that produced simultaneously by vitamin D in comparable rats, three litters were treated with procedure (*c*). The results in the animals transferred to diet 1·9 were the same as those above.

The line test in the animals getting vitamin D did not become positive till after 6 days in two of the litters and after 4 days in the other. The changes in the teeth after vitamin D were the same as previously described (Irving, 1944*a*), the teeth of one of the litters reacting after 2 days and those of the other two after 1 day. While the reactions after diet 1·9 on the one hand, and vitamin D on the other, were similar in some respects, they differed in several important details. In particular, the reaction in the intermediate and proximal predentin differed considerably. After vitamin D, the first reaction in this part of the tooth, seen after 4 days, was a very fine peppering of calcospherites in the predentin, which faded off towards the old and already calcified dentin (Pl. 1, fig. 6). By 6 days, the calcospherites had fused to form a fine stripe at normal predentin width from the odontoblasts (Pl. 2, fig. 7). There was, however, no coarse mottling of large calcospherites between the stripe and the already calcified dentin. The space was either uncalcified, or else contained a fine sprinkling of very small calcospherites. In addition, the reaction in this part of the tooth was much slower after vitamin D than after diet 1·9.

Three litters of rats were treated with procedure (*d*). This experiment was undertaken to investigate the very early changes in the teeth and to find out if the teeth reacted before 24 hr. had elapsed after changing the diet, as appeared probable from earlier observations. One rat of each litter was killed after 6 hr., and two thereafter at 6-hourly intervals up to 30 hr. The line test became positive in one litter after 18 hr., in one after 24 hr., and not at all in the third litter. Kramer *et al.* (1931) and Karelitz & Shohl (1927) have noted that healing may occur in rachitic bones from 12 to 24 hr. after the addition of P to the diet. In all cases but one, a fine hypercalcified stripe occurred after 12 hr. in the distal area on both sides of the tooth. On the labial side this stripe was laid down at about 16 μ from the odontoblasts. With the passage of time, this stripe became wider and more distinct and also extended farther and farther proximally. After 24 hr. this stripe on the labial side joined proximally with a line of calcospherites which were laid down in an irregular fashion and which were the precursors of those which later filled this area in a haphazard manner. These results showed that the previous conclusion was correct, namely, that the tooth reacted within 24 hr. after the change of diet.

Animals transferred from diet 5·6 *to diets* 3·7, 3·0 *or* 2·5

It was thought that the differences noted between the action of vitamin D and that of diet 1·9 might be due to the fact that the Ca : P ratio of the diet had been so suddenly and so markedly lowered. Diets of Ca : P ratio intermediate between 1·9 and 5·6 were therefore used, the total content of Ca not being altered (procedure (*e*)). Nine litters of rats were treated in this way, five litters being transferred to diet 3·7, two litters to diet 3·0 and two litters to diet 2·5, all after 28 days on diet 5·6. In some cases the animals were

weighed after transfer to the new diets, and in none was weight lost; in most an increase was found. A careful note was also taken of the food consumption, and no diminution in the amount of food consumed was seen after the change.

Two rats from each of two of the litters transferred to diet 3·7 were killed on the 1st, 2nd, 3rd or 4th day after transference. The results showed that this was rather too short a period of observation, so one rat of each of the remaining litters was killed on the 2nd to 8th days after changing to diet 3·7. The line test in the first two litters had not become positive by the 4th day. In the remaining three litters, the line test had become positive after 8, 6 and 7 days respectively. The healing was still of the same abnormal kind and could not be classified into the ordinary line-test stages. In no case was advanced healing found, even at the end of the experimental period; the most that was seen was a complete line across the epiphysis, with downgrowths joining it to the diaphysis, which somewhat resembled stage 3 of healing.

The response in the teeth was exactly like that described above under procedure (*b*), with the exception that the intensity of the reaction, as judged by the staining of the newly calcified dentin, was much less marked. The teeth in all cases reacted within 24 hr. by laying down a distal stripe on both lingual and labial sides. On the labial side the same interglobular dentin response was seen, but in some litters it took rather longer to develop than when the Ca : P ratio of the diet was changed more drastically. Pl. 2, fig. 8, shows the building up of interglobular dentin in the tooth of an animal after 4 days on diet 3·7. The reaction was just as with procedure (*b*). In later stages the whole area filled up with interglobular dentin, but the predentin was still abnormally wide at the end of the experimental period.

The action of diet 3·7 thus resembled that of vitamin D in postponing the time of epiphyseal reaction, for it had been previously noted (Irving, 1944*a*) that at least 4 days separated the time of reaction of the teeth and bones after vitamin D dosage. This was the only particular in which a resemblance was seen; the reaction in the teeth bore little resemblance to that caused by vitamin D.

Of the animals on diets 3·0 and 2·5, two from each litter were killed after 1, 2, 3 or 4 days on the new diet. The line test became positive after 2 days in one litter transferred to diet 3·0 and after 4 days in the other litter; with diet 2·5, the line test became positive after 2 days in one litter and 1 day in the other. Thus, as with diet 3·7, the time of line-test reaction was postponed with diet 3·0, compared to the reaction time with diets 1·9 or 2·5, becoming earlier as the Ca : P ratio of the diet was made more favourable. In all cases, however, the line test had the same peculiar character previously described, and was not like that after vitamin D dosage. The changes in the teeth in the two groups were identical, and very similar to those seen when the animals were transferred to diet 1·9. With both, the teeth reacted within

24 hr. of changing the diets. The pattern of change was exactly like that previously described, and, in particular, the coarse interglobular response in the proximal part of the tooth still occurred.

It was therefore concluded from these observations that the difference between the action of vitamin D on the teeth, and probably also on the bones, and that of lowering of the Ca : P ratio of the diet, was not due to the sudden and considerable change in the dietary ratio.

Animals transferred from diet 0·26 *to diet* 1·9

All the animals gained weight over the period on diet 0·26. Three rats lost weight after transference to diet 1·9 (procedure (*b*)). The results obtained from these animals were the same as those from animals whose weight increased. Shohl & Wolbach (1936) found that some rats on diets with a Ca : P ratio of 0·25 showed carpopedal spasms and tremors. The animals on diet 0·26 in the present work were somewhat more excitable than normal, but no changes resembling tetany were seen.

As was previously stated (Irving, 1944*a*), it was also found here that none of the animals on diet 0·26 had widened epiphyseal cartilages, and so it was not possible to assess the effects of changes in the diet on the bones by means of the line test. One litter of rats was treated with procedure (*a*). The control animals had very badly calcified teeth and many vascular inclusions in the predentin. The animals which had been transferred to diet 1·9 showed a marked response in the teeth; this took the form of a broad hypercalcified stripe on the distal lingual and labial sides, which was often laid down in incremental lines. From measurements of this stripe it was found that the teeth had reacted to the change in diet within 24 hr. In the intermediate and proximal parts of the labial side the response became more of an interglobular dentin type, with large calcospherites scattered haphazard throughout the predentin. These tended to coalesce on the odontoblast side to form a stripe which fused distally with the solid stripe already mentioned (Plate 2, fig. 9). The reaction was broken by a series of 'dips' where calcification did not occur, or only occurred to a slight extent; vascular inclusions were found in these parts of the predentin, and apparently inhibited calcification. This inhibition of calcification in the proximity of vascular inclusions has also been noted by Schour, Chandler & Tweedy (1937), who stated that an arcade-like appearance was caused. The response after diet 0·26 was, in general, identical with that already found in animals originally on diet 5·6, except for the arcades in the proximal part of the tooth. In the case of animals on diet 5·6, there were many fewer vascular inclusions and so the arcades were not a prominent feature.

Three litters of rats were treated with procedure (*b*). One day after the change of diet the teeth already showed a fine reactive stripe in the distal part and on both lingual and labial sides. With the passage of time the stripe

on the lingual side extended over the whole length of the tooth and thickened, often being laid down in incremental lines. On the labial side, the stripe also thickened in an incremental way and extended proximally. As it did this, a haphazard scattering of calcospherites developed in the intermediate and proximal predentin, fusing on the odontoblast side to form an irregular stripe, which joined with the distal stripe. In early stages, these calcospherites appeared first near the calcified dentin, and then built up towards the odontoblast side (Pl. 2, fig. 10). The arcades already mentioned occurred frequently; but apart from these, which were caused by the large number of vascular inclusions, the reaction in these animals appeared identical with that in the animals first on diet 5·6.

Three litters of rats were treated with procedure (*c*). The teeth of the animals transferred to diet 1·9 reacted in the same way as described in the preceding paragraph. The teeth of the animals given vitamin D behaved as previously described (Irving, 1944*a*), save that, in the present results, a distal stripe was more prominent on the labial side. One day after vitamin D dosage a fine stripe was visible on the lingual and labial sides in the distal part. On the lingual side this extended proximally with the passage of time and became wider. On the labial side an irregular mass of calcospherites developed in the intermediate and proximal predentin after about 4 days (Pl. 2, fig. 11). The distal stripe extended back in later days and blended with these calcospherites. This stripe showed the same arcades as were noted in the other teeth. The reaction in the proximal part of the tooth developed more quickly after vitamin D than after diet 1·9, being, after 6 days, about 2 days more advanced. Apart from this, the reaction after vitamin D was identical with that after changing to diet 1·9. In the previous report (Irving, 1944*a*) the animals were given 18·4 i.u. of vitamin D, whereas here they had 30 i.u. This accounts for the earlier response of the teeth.

Two litters of rats were treated with procedure (*d*), as it appeared from some of the above results that the teeth had reacted within 24 hr. of the change of diet. This supposition was found to be correct. The lingual side of the tooth reacted 12 hr. after the change of diet; the labial side reacted after 18 hr. in one litter and after 24 hr. in the other. The reaction in both cases was a very narrow hypercalcified stripe seen in the distal area. On the labial side this first appeared at a distance of 9μ from the odontoblasts. This low figure, compared with that of 16μ obtained when animals were transferred from diet 5·6 to diet 1·9 under similar conditions, is presumably due to the very slow incremental growth rate of the dentin when animals are on diet 0·26 (Irving, 1944*a*).

Since the reaction caused by diet 1·9 resembled so closely that caused by vitamin D after diet 0·26, it was not thought necessary to analyse the reactions further by using procedure (*e*).

Discussion

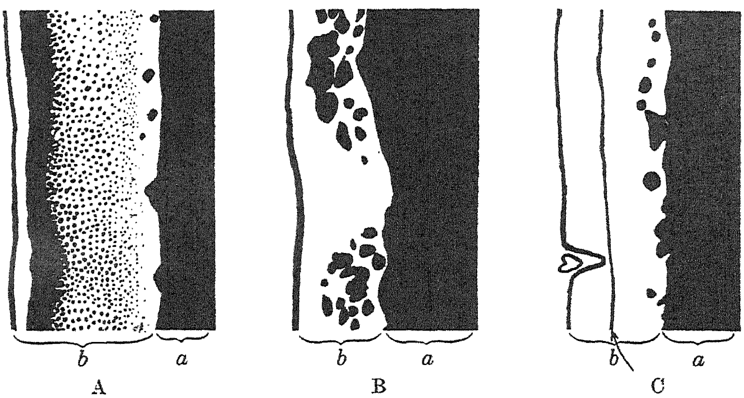
Several writers (McCollum, Simmonds, Shipley & Park, 1922*a*; Kramer *et al.* 1931, and others) have drawn attention to the fact that changes in the diet or treatment of rachitic rats may cause spontaneous healing of rickets to take place. This is usually attributed to a reduction in the food intake causing a loss of weight. From the data cited above it can be concluded that loss of weight was not responsible for the changes described in the present results.

Shohl, Bennett & Weed (1928) have shown that variations in the acidity or alkalinity of a rachitogenic diet, concurrent with the addition of extra P, can affect the response of the animals. The alkalinity of the diets used in the present work was calculated by the method of Sherman & Gettler (1912), save that a valence of 1·8 was attributed to PO_4, as was done by Shohl *et al.* (1928), since this is its neutralizing value at the reaction of the body. Calculations showed that diet 1·9 had an alkalinity equivalent to 640 c.c. of 0·1 N. alkali per 100 g., and for diet 5·6 the figure was 600 c.c. of 0·1 N. alkali. Diets 3·7, 3·0 and 2·5 had values intermediate between these two figures. Since these differences were so slight, it was felt that the changes induced by these diets could not be attributed to alterations in the alkalinity of the diets. Shohl *et al.* (1928) found that the changes caused by extra P were virtually the same if the alkalinity of the diet was 859 or 311 c.c. of 0·1 N. alkali per 100 g. Diet 0·26 had a much lower alkalinity, equivalent to 93 c.c. of 0·1 N. alkali per 100 g. However, since the effects produced when changing from this diet to diet 1·9 were the same as those found when continuing the animals on diet 0·26 but dosing them with vitamin D, it was again felt that the change in reaction of the diet had little to do with the observed effects.

The retrospective type of calcification of predentin, previously described as caused by vitamin D in animals on low Ca : P ratio diets (Irving, 1944*a*), also occurs after both high and low Ca : P ratio diets if the Ca and P contents are adjusted to a favourable ratio. The previously calcified dentin is not changed, but the process of calcification which is slowly going on is accelerated and extends to predentin which is nearer to the odontoblasts. How and why this should happen it is at present impossible to say, nor is it easy to picture the mechanisms involved. Presumably the elements concerned in calcification get to the site to be calcified via Tomes's fibres (Lefkowitz, 1943), and, on high or low Ca : P ratio diets, this process is so slow that the incremental growth rate outstrips that of calcification, thus causing wide predentin. This suggestion has been made by Schour & Hoffman (1939). On adjusting the diet, a better supply of these elements is available and in larger amount, and thus this process of calcification is accelerated, predentin laid down as much as 6 to 8 days previously (calculated from Irving, 1944*a*) being retrospectively calcified. Vitamin D, acting in conjunction with low Ca : P ratio diets, does the same.

Why the vitamin acting on animals taking high Ca : P ratio diets should not do this, but cause the odontoblasts to begin forming normal predentin anew, leaving the older predentin almost untouched, is quite unexplained.

In Text-fig. 1 are shown the three different types of recalcification of malformed predentin which have so far been described. In A is shown that which occurs with high Ca : P ratio diets after vitamin D and in parathyroidectomized rats after vitamin D or parathormone (Schour, Tweedy, Chandler & Engel, 1937). The action of vitamin D on rachitic rats also causes a healing reaction in the epiphyses as shown by the line test. In B is shown the reaction



Text-fig. 1. Camera lucida drawings to illustrate the various types of response in abnormal predentin. a = dentin, b = predentin. The odontoblasts have not been drawn. The diet numbers refer to the Ca : P ratio. A. Animal on diet 5·6. Given 30 i.u. of vitamin D 6 days previously. The newly calcified area in the predentin, backed by very fine calcospherites, is shown. B. Animal originally on diet 0·26, changed 4 days previously to diet 1·9. The typical coarse interglobular dentin response is seen. A similar change would have occurred had the animal been dosed with vitamin D, or if it had originally been on diet 5·6 and then changed to diet 1·9. C. Animal on diet 0·26 injected with NaF 4 days previously. The arrow points to the hypercalcified line. The indentation in the predentin is a vascular inclusion.

described in the present paper, when the Ca : P ratio of the diets is changed, or vitamin D is given to rats on low Ca : P ratio diets. This is accompanied by abnormal epiphyseal healing if the animal is on the high Ca : P ratio diet. In C is shown the change that occurs in animals on low Ca : P ratio diets if F or P is injected (Irving, 1943, 1944*b*). This is a very short-lived process in which again only the predentin forming at the moment of injection is affected.

In all cases where the Ca : P ratio of the first diet was high and that of the second diet was lower or normal, the early stages of the healing process in the epiphyses were not like those in the classical line-test response but fell into the class described as diaphyseal healing by Bills, Honeywell, Wirick & Nussmeier (1931). Such calcification may occur after D medication, but preliminary work in this department (Irving, 1944*a*) has shown that vitamin D causes

it only in animals with insufficiently developed rickets, as has also been found by McCollum *et al.* (1922*b*), Bourdillon, Bruce, Fischmann & Webster (1931), and others. Since all the animals used in the present experiments were strictly comparable as regards the severity of the rickets, and all those dosed with vitamin D showed proper lines, it would appear that this response on adding P to the high Ca : P ratio diet normally occurs after such a procedure. This supposition is supported by the work of Nicolaysen & Jansen (1939) who found that the histological structure of the bones of rachitic rats may differ after the giving of P from that which is found after vitamin D medication.

In the previous paper (Irving, 1944*a*) it was noted that, after vitamin D dosage, the bones did not respond till at least 4 days had elapsed after the teeth had reacted. It is apparent from the results above that this difference in reaction can be imitated by varying the Ca : P ratio of the second diet. The more unfavourable the ratio of the second diet, the later does the bone react as compared with the tooth. This is what would be expected from previous work (Gaunt, Irving & Thomson, 1939), in which it was shown that under unfavourable conditions for calcification, the teeth were less affected than the bones. The effect of varying the Ca : P ratio upon the healing of rickets has also been studied by Coward & Kassner (1940), who found that the amount of healing in the epiphyses of rachitic rats gradually increased as the Ca : P ratio of the diet was lowered. Animals which had become rachitic after a period on a rachitogenic diet were used; subsequently, after 10 days on a diet with a Ca : P ratio of 3·84 or 3·69, no healing was found, but with a diet of Ca : P ratio 3·43, an average degree of healing of 0·08 occurred. With lower Ca : P ratios, still better healing was found. It seems likely (Irving, 1941) that epiphyses, which show a lower grade of healing after a definite period, have begun to recalcify later than those which have a higher grade of healing. The present results with diet 3·7 are an interesting confirmation of the findings of Gaunt & Irving (1940), that normal teeth were formed on diets with Ca : P ratios between 4 and 0·5, provided that the Ca and P contents were each at least 0·3 %. Diet 3·7 caused healing of the teeth in spite of the Ca : P ratio of 3·7, since the P content was 0·4 %.

It would appear from the above findings that the early stages in the healing process in the teeth, and probably also in the epiphyses, of animals on high Ca : P ratio diets, differ when vitamin D is given from those which occur when the Ca : P ratio of the diet is adjusted to a normal figure. Kramer *et al.* (1931) have shown that while vitamin D causes the blood P to rise in rachitic rats, lowering the Ca : P ratio of the diet by adding extra P is followed by a much greater and quicker increase in blood P. The experiments quoted above make it unlikely that the differences in the action of the vitamin, and of the adjustment of the Ca : P ratio, were due to the rather drastic change in the latter, since the same differences existed when the Ca : P ratio was altered much

more slightly. In animals on low Ca : P ratio diets, vitamin D caused a healing change in the teeth identical with that found after the Ca : P ratio of the diet was raised to normal. It would appear at least from the evidence of changes in the teeth, that the underlying mechanisms of the action of vitamin D, and of the adjustment of the Ca : P ratio of the diet, are not identical when the Ca : P ratio is initially high; but that they may be the same when the Ca : P ratio of the diet is initially low.

SUMMARY

1. Young rats weighing from 50 to 60 g. were placed on rachitogenic diets of high or low Ca : P ratio for 28 days. Some were then transferred to diets of more normal Ca : P ratio and others were dosed with vitamin D. After a further period, they were killed and their incisor teeth and their bones examined. The histological response in the dentin was such that the time and nature of the action of the new diet or of vitamin D could be compared.

2. Some of the rats on the diet with a Ca : P ratio of 5·6 and showing marked rickets, were transferred to a diet with a Ca : P ratio of 1·9. Healing in the bones was first detected from 18 hr. to 2 days after change of diet, but the pattern of healing was abnormal and unlike that after vitamin D dosage. A reactive response was seen in the teeth after 12 hr. This response, especially in the early stages, was unlike that caused by vitamin D, being an irregular deposit of interglobular dentin affecting the matrix laid down as much as 6–8 days before the diet was changed.

3. Others of the rats on the diet with a Ca : P ratio of 5·6 and showing marked rickets, were transferred to diets with Ca : P ratios of 2·5, 3·0 or 3·7. The time of the line response in the bones was the more delayed the higher the Ca : P ratio, averaging $1\frac{1}{2}$, 3 and 6 days respectively after change of diet. The response was of the same abnormal nature. The changes in the teeth were qualitatively the same as those noted in (2) above, and began in all cases within 24 hr. of the change of diet. It was concluded that the differences in the action of vitamin D and of the adjustment of the Ca : P ratio of the diet mentioned in (2) above, were not due to the considerable change in the Ca : P ratio of the diet.

4. Rats on the diet with a Ca : P ratio of 0·26 were transferred to a diet with a Ca : P ratio of 1·9. As is usual with low Ca : P ratio diets, no widening of the epiphyses was seen. The teeth responded 18 hr. after the change of diet, the appearance of the new calcification closely resembling that after vitamin D dosage.

5. The changes in the teeth of animals initially on high or low Ca : P ratio diets appeared identical when the ratio of the diets was adjusted to a normal one. Vitamin D produced a similar histological change in the teeth of animals on the low Ca : P ratio diet, but a different response in animals on the high Ca : P ratio diet.

The writer is indebted to Dr Helen Schwartz, Department of Chemistry, University of Cape Town, for assistance with the dosing of the animals, and for analysing the diets; to Prof. R. Goetz for taking the microphotographs; and to Mr D. G. Duncan for technical assistance. The expenses of this work were defrayed by grants from the Staff Research Fund, University of Cape Town, and the National Research Council and Board, Union of South Africa; for these, grateful acknowledgement is made.

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EXPLANATION OF PLATES 1–3

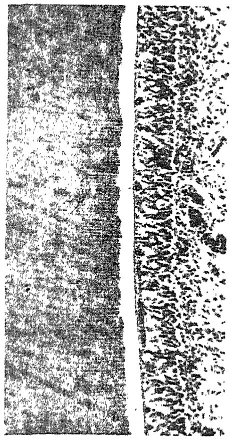
Figs. 2 to 11 are longitudinal sections of the intermediate part of the labial side of the upper incisor tooth. Magnification in all is ×90. a = dentin, b = predentin, c = odontoblasts, d = pulp. The diet numbers refer to the Ca : P ratio.

Plate 1

Fig. 2. Normal tooth to show the various structures.

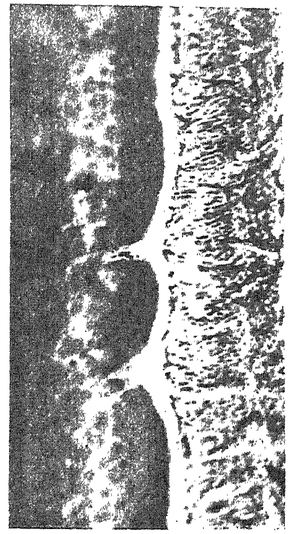
Fig. 3. Section from an animal on diet 5·6 for 28 days and transferred to diet 1·9 for 10 days. a^1 = dentin formed while on diet 5·6; a^2 = dentin formed while on diet 1·9. Two arcades are shown. These were not very common in animals on diet 5·6.

Fig. 4. Section from an animal on diet 5·6 for 28 days and killed 2 days after transfer to diet 1·9. Note the formation of coarse calcospherites in the predentin.



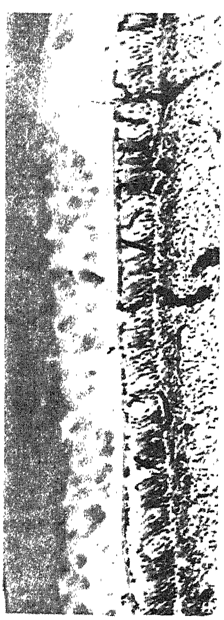
a b c d

Fig. 2.



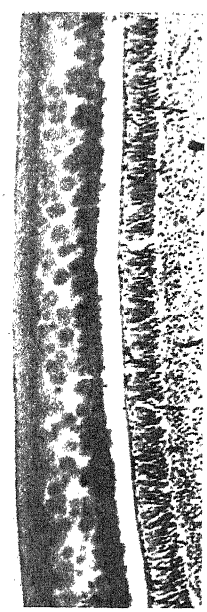
a^1 a^2 b c

Fig. 3.



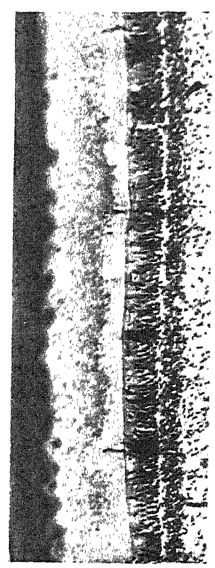
a b c

Fig. 4.



a^1 a^2 b c

Fig. 5.



a b c

Fig. 6.

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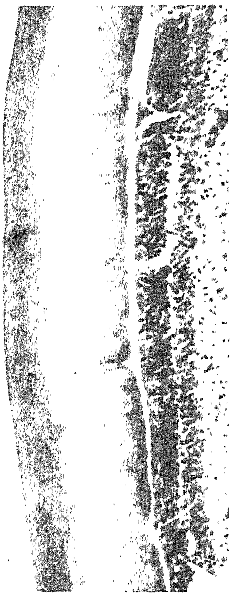
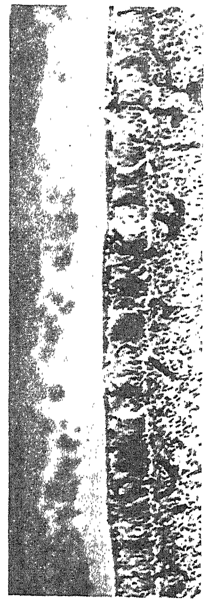


a b c

Fig. 7

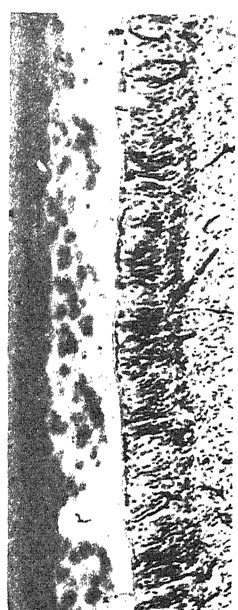


a b c

Fig. 8.

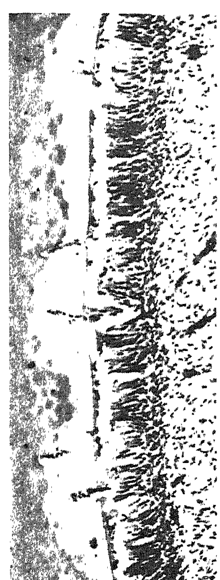
a^1 a^2 b c

Fig. 9.



a b c

Fig. 10.



a b c

Fig. 11.

Fig. 13.

Fig. 12.

Fig. 5. Section from an animal on diet 5·6 for 28 days and killed 6 days after transference to diet 1·9. a^1 = dentin formed while on diet 5·6; a^2 = the interglobular dentin and calcified stripe formed under the influence of diet 1·9.

Fig. 6. Section from an animal 32 days on diet 5·6, having been dosed with 30 i.u. of vitamin D 4 days before being killed. Note the fine peppering of calcospherites in the predentin.

PLATE 2

Fig. 7. Section from an animal 34 days on diet 5·6, and which received 30 i.u. of vitamin D 6 days before being killed. Note the narrow line of new dentin which has formed in the predentin.

Fig. 8. Section from an animal 28 days on diet 5·6 and then transferred to diet 3·7 for 4 days. This section shows the interglobular dentin response in the predentin caused by diet 3·7.

Fig. 9. Section from an animal 28 days on diet 0·26 and transferred to diet 1·9 10 days before being killed. a^1 = dentin formed while on diet 0·26; a^2 = dentin and interglobular dentin formed while on diet 1·9. The typical arcades are well shown.

Fig. 10. Section from an animal 28 days on diet 0·26 and killed 5 days after transfer to diet 1·9. The second diet has caused the formation of large calcospherites in the predentin. Compare with Pl. 1, fig. 4.

Fig. 11. Section from an animal on diet 0·26 for 32 days and given 30 i.u. of vitamin D 4 days before being killed. Note the interglobular dentin response in the predentin, similar to that seen in Figs. 4 and 10.

PLATE 3

Figs. 12 and 13 are longitudinal frozen sections of the lower ends of undecalcified ulnae and radii, treated with $AgNO_3$. Magnification ×12·5.

Fig. 12. Typical early line-test response in a rachitic rat after treatment with vitamin D.

Fig. 13. Abnormal epiphyseal response in a rachitic rat caused by transference to diet 3·7.

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INTERACTION OF DRUGS AND THE EFFECT OF COOLING ON THE ISOLATED MAMMALIAN INTESTINE

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This paper describes certain changes in the rhythmic activity and pharmacological properties of the mammalian intestine, which are produced by cooling to about 1° C. The effects of cooling have been previously studied on the crop and gizzard of the earthworm (Ambache, Dixon & Wright, 1945), and similar changes were observed which were attributed to a loss of function in the enteric plexus. From such experiments it appeared that muscle fibres are able to withstand long periods of cooling; indeed, Macht (1914) has recorded responses to adrenaline from strips of human pulmonary artery cooled for a total of 49 days. Acetylcholine synthesis in the enteric plexus is, on the other hand, abolished after 4 or 5 days (Dikshit, 1938). The method of cooling provides, therefore, a means of obtaining an intestinal smooth muscle preparation which is apparently free from the influence of the nerves within it. In the experiments on the earthworm, it was possible, in this way, to make a clear distinction between substances which act on the muscle fibres directly, such as acetylcholine and adrenaline, and other substances, e.g. KCl and $BaCl_2$, which appear, normally, to release acetylcholine at the nerve-endings and so indirectly cause the muscle to contract. For, whereas the action of the substances in the former category was retained after cooling, the action of those in the latter, i.e. those which act indirectly, was lost. The same distinction could be made by treating the gut with an excess of calcium, which appears to prevent the release of acetylcholine by substances of the second category (cf. Brown & Feldberg, 1936). In addition, it was shown that the augmentor action of these substances was enhanced by eserine.

In deciding whether a drug acts on the gut directly or not this method may supply information which cannot be provided by nicotine or by atropine.

It is known, for instance, that nicotine, although it paralyses autonomic ganglion cells, does not prevent the release of acetylcholine from cholinergic nerve-endings (Feldberg & Vartiainen, 1934).

As regards atropine, although it inhibits 'added' acetylcholine, it is unable, in the gut, to interrupt the transmission, on the one hand, of vagal impulses and peristaltic reflexes (Bayliss & Starling, 1899) and, on the other, of impulses arising from the pharmacological stimulation of

ganglion cells (Vogt, 1943). In order to explain this discrepancy, Dale & Gaddum (1930) have suggested that the nerve-endings concerned liberate acetylcholine in such close proximity to the receptive mechanism in the muscle that 'atropine cannot prevent its access thereto'. If, indeed, these nerve-endings are 'wholly within the barrier' (of atropine), and should substances exist which are able to release acetylcholine at these endings, then it follows that these substances too would fail to be inhibited by atropine. Doubts have been raised, also, as to the specificity of atropine, by Mellanby & Pratt (1939, 1940), who believe that part at least of its paralysing action is due to a direct effect on the muscle substance itself. This is more likely to occur with large doses of atropine. For these reasons, it seemed desirable to re-examine, in mammals, the action of various drugs previously subjected to the atropine test. In search for further confirmation of the main conclusions arrived at by this method, experiments were performed to find out whether the drugs appearing to act indirectly do, in fact, release acetylcholine from the intestine.

METHODS

Preparations of the isolated small intestine (jejunum or ileum) were made, in the usual way, from rabbits, guinea-pigs and mice, freshly killed by concussion. After a preliminary wash-out, the lumen was tied at both ends and an inch of gut was suspended in oxygenated Tyrode's solution of low magnesium content (0–0·01 % $MgCl_2$) at 36–38° C. The bath volume was 5 c.c. unless otherwise stated, and the fluid was renewed by overflow. The drugs were added with a syringe, the volume injected varying between 0·05 and 0·2 c.c. When electrical stimulation was used, the arrangement of the bath (10 c.c.) and non-polarizable electrodes was the same as that described by Prasad (1935). The muscle was stimulated for $\frac{1}{5}$ sec. every 10 sec. by an alternating current (50 cyc./sec.) at 21 or 54 V. The current between the electrodes was measured and found to be 0·18 and 0·48 amp. respectively.

In the experiments on cooling, each preparation was pharmacologically examined when fresh, and was then kept in the refrigerator in Tyrode's solution at 0–2° C. After a varying number of hours in the cold, the muscle was replaced in the oxygenated bath and allowed to recover at 36–38° C. for $\frac{1}{2}$–$\frac{3}{4}$ hr. before it was re-examined.

In the experiments on the mouse, the same sensitive 'balance-type' lever was used as described previously (Ambache *et al.* 1945). This lever was also used to record the weak movements of the rabbit's intestine seen in the final stages of cooling.

To demonstrate releases of acetylcholine by various drugs, isolated strips of small intestine were incubated with each drug in a warm oxygenated bath. For such experiments the guinea-pig was chosen because, in this animal, the level of acetylcholine synthesis is about twice as high as in most other laboratory animals (Dikshit, 1938). In the earlier experiments, the amount of acetylcholine liberated by the gut into the surrounding bath fluid (containing eserine) was determined (by assay on the frog's rectus abdominis muscle) at 10 min. intervals, before and after the addition of drugs. In later experiments, the whole acetylcholine contained in the gut and bath fluid was determined in each case. In this method, the pooled small intestines of two freshly killed guinea-pigs were slit open by a longitudinal incision and thoroughly rinsed with a bicarbonate-free Locke's solution; the gut was then divided up into equal strips, 13–15 cm. in length, and placed in two beakers, alternate strips to each beaker. Each set of strips was then dried between filter-papers and weighed rapidly. The two lots, each weighing between 10 and 16 g., were then incubated separately for 30–40 min. at 36–38° C. in 6 c.c. of oxygenated Locke's solution (free from bicarbonate) to which was added 0·04–0·3 c.c. of 1/200 eserine sulphate. During the incubation period, the particular drug under investigation was introduced at various intervals into one of the beakers. The other strips, which received no drugs, served as a control.

After incubation, the total amount of acetylcholine present in the bath fluid and the tissue was determined, in each case, by a method of acid extraction and boiling, which was essentially the same as that described by Feldberg (1943); the same precautions were taken during the assay on the frog's rectus abdominis muscle.

RESULTS

The spontaneous activity of the isolated intestine

Fresh preparations

Rhythmic activity is usually poor in fresh preparations of the guinea-pig's ileum. This section deals, therefore, with the intestines of rabbits and mice, which exhibit regular rhythmic contractions. Fresh rabbit preparations possess a simple rhythm consisting of vigorous pendulum movements at a frequency varying between 8 and 12/min. The rhythmic activity of the mouse's

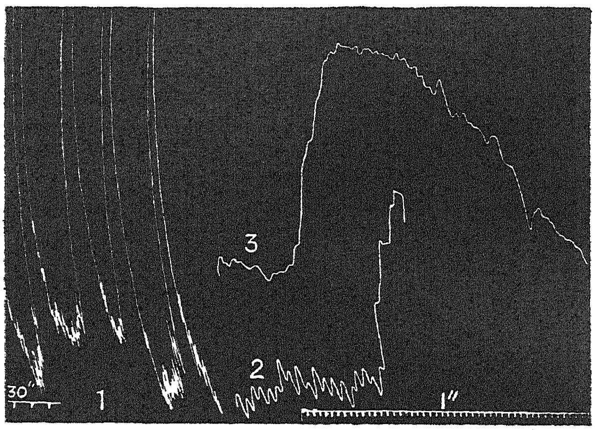

Fig. 1. The normal activity of the isolated small intestine of the mouse in the presence of a 'liquid bolus'. Contraction upwards in these and subsequent tracings. Magnification × 13. Records taken with slow (1) and fast (2, 3) drums. 1, small pendulum movements and four large peristaltic waves; 2, pendulum movements, frequency 45/min.; 3, a peristaltic wave lasting 48 sec. Time in 30 sec. for 1, and 1 sec. for 2 and 3.

ileum is somewhat different (Fig. 1). It consists of two components: there is, first, a continuous background of small, regular contractions, which are pendulum movements of relatively high frequency (40–45/min.); and secondly, superimposed on this rhythm, are large waves of contraction, each lasting from ½ to 1 min.; they are connected with peristalsis and will be referred to as such. They are abolished by nicotine and by short periods of oxygen-lack, leaving the background of pendulum movements unchanged. The stimulus for them appears to be the distension of the lumen by a 'liquid bolus' of Tyrode's solution entrapped in the intestine by the ligatures at its ends.

With the exception of one guinea-pig preparation (Fig. 12 C), peristalsis was not observed in the intestines of rabbits or guinea-pigs under the present conditions of experiment. But in subsequent experiments, in which greater distension was used and the oxygen bubbling through the bath was replaced by expired air, peristalsis was present. The effect of expired air, besides

buffering the Tyrode's solution, is to shift its pH slightly to the acid side, and this would have an effect on acetylcholine transmission like that of eserine, as demonstrated by Gesell, Brassfield & Hamilton (1942).

Effect of cooling on spontaneous activity

Peristalsis. Cooling for 18–24 hr. abolishes the normal peristaltic activity of the mouse gut, without affecting pendulum movements except for a slight slowing (Fig. 2). At this stage the ganglion cells still respond to nicotine, and the loss of peristalsis would seem to be due to a failure of transmission within Auerbach's plexus.

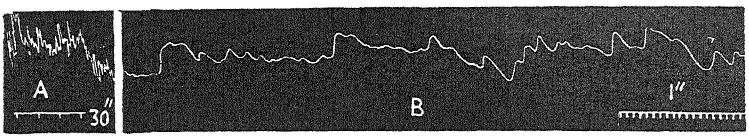

Fig. 2. Mouse ileum cooled for 19½ hr. at 2½–4° C. Lumen distended with liquid paraffin. Peristalsis absent throughout the experiment. Pendulum movements recorded with slow (A) and fast (B) drums; frequency 18–25/min. Time in 30 sec. for A, and 1 sec. for B.

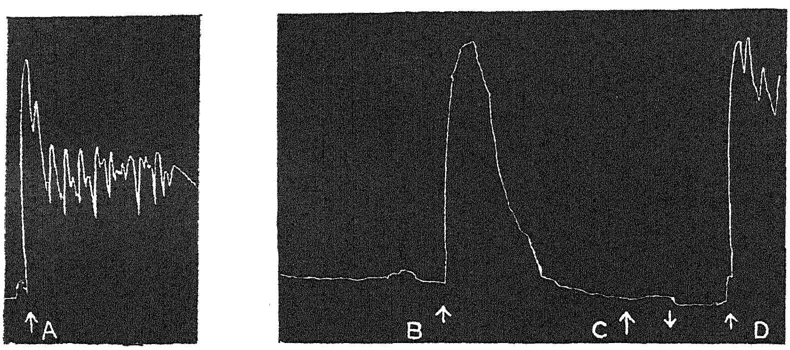

Fig. 3. Rabbit duodenum, cooled for 8 days at 0–2° C. Magnification × 20. No rhythmic activity. Effect of electrical stimulation at A (54 V.; shocks applied at 10 sec. intervals) and at D (four shocks). At B, 20 μg. of acetylcholine-HCl. No response to 2 mg. $BaCl_2$ at C.

Pendulum movements. The effect of cooling on pendulum movements was investigated in the rabbit only, because of their greater amplitude in this animal. They were finally extinguished after 4 or 5 days. Before this, the movements became progressively weaker and slower, and, in order to show them up, it was necessary to reduce the load on the muscle in proportion to the period of cooling. After 4 or 5 days, no movement whatsoever could be detected with the sensitive lever, even with the writing-point off the drum. At this stage, however, the muscle fibres still retained their excitability to electrical stimulation (Fig. 3) and to acetylcholine (Figs. 3, 9 and 10).

THE ACTION OF DRUGS

This section deals with the changes produced by cooling in the response to drugs. In addition, it describes the interaction of the various drugs in an attempt to elucidate the mechanism of their action.

Acetylcholine

The following observations show that acetylcholine belongs to both the 'direct' and 'indirect' categories of drugs, since, apart from its 'muscarine action' on the muscle fibres, it also has a 'nicotine action' on the ganglion cells. This distinction is again apparent in later sections.

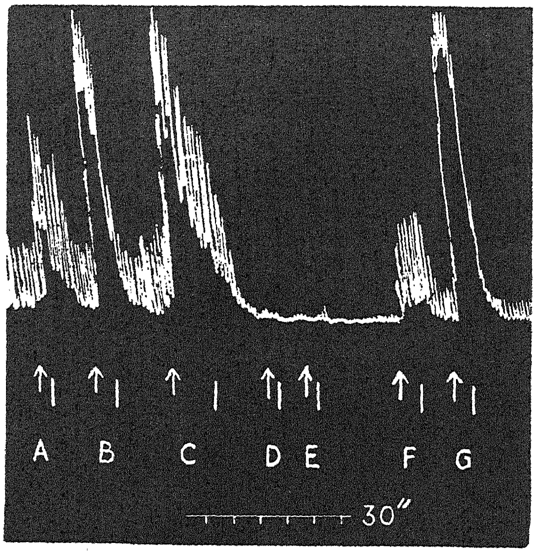

Fig. 4. Fresh rabbit's jejunum. Magnification × 6. Time in 30 sec. A, 0·1 μg. acetylcholine-HCl; B, E, 0·2 μg. acetylcholine-HCl; C, 10 μg. nicotine hydrogen tartrate; D, 100 μg. nicotine hydrogen tartrate; F, 1 μg. acetylcholine-HCl; G, 2 μg. acetylcholine-HCl.

Fresh intestine. The existence of these two effects can be shown, in fresh preparations, by comparing the thresholds for acetylcholine before and after paralysis of the ganglion cells by nicotine. This is illustrated in Fig. 4, where the initial response of a rabbit jejunum to 0·1 and 0·2 μg., respectively, of acetylcholine is shown at A and B. Nicotine (10 μg.) was then introduced into the bath at C, after which a further dose of 100 μg. of nicotine was ineffective, at D, indicating paralysis of the ganglion cells. After changing the bath fluid, 0·2 μg. of acetylcholine (at E) had no effect; the dose of acetylcholine was therefore increased to 1 μg., which produced the contraction at F, rather smaller than A. The effect, at G, of 2 μg. was approximately equal to that of 0·2 μg., originally, at B.

In another experiment, on a guinea-pig's ileum, the threshold concentration for the smallest noticeable response to acetylcholine was 1 in 10^9 originally, and 1 in 10^7 after nicotine paralysis.

These changes in threshold after nicotine are of the same order as those observed by van Esveld (1928) in circular muscle preparations, after the mechanical removal of ganglion cells.

Cooled intestine. Acetylcholine, by virtue of its 'muscarine action', causes large contractions in cooled preparations, long after peristaltic reflexes and pendulum movements have disappeared (Figs. 3, 9 and 10).

Eserine

Fresh preparations. Since acetylcholine transmission occurs at two different sites in the intestine, it was to be expected that eserine would have a twofold action on the intestine: one, by preserving the acetylcholine at the preganglionic nerve-endings in Auerbach's plexus, and another depending upon the preservation of acetylcholine at the cholinergic nerve-endings in relation to the muscle fibres. For the sake of a better understanding of the changes produced by cooling it is necessary to distinguish clearly between these two effects.

The first action manifests itself in an enhancement of peristalsis; this effect is particularly striking in those rabbit preparations in which peristaltic activity is normally in abeyance, as shown in Fig. 5. In this experiment a moderate distension of the lumen, by the saline enclosed within it, was clearly visible through the side of the bath. Nevertheless, the initial activity of the gut consisted entirely of pendulum movements. As observed by Feldberg & Solandt (1942, Fig. 4 and text on p. 145), the main effect of eserine, in such preparations, consists in large waves of contraction in the circular muscle, which produce corresponding waves of passive relaxation in the longitudinal layer. Two such waves are shown in Fig. 5 A, beginning $\frac{1}{2}$ min. after the eserine (1 μg.) was washed out. A much stronger effect, consisting of five large waves, is shown at P, starting 45 sec. after a mixture of eserine (1 μg. at C) and calcium (at D) was washed out (B shows that calcium alone had no such effect; this action of calcium is discussed later, in the section on calcium). The size and duration (1–$1\frac{1}{2}$ min.) of these waves, and the fact that they are abolished by nicotine, at H, suggest that they are peristaltic contractions. Such an effect of eserine, in facilitating latent peristaltic reflexes, could be explained by an improvement in transmission in the synapses of Auerbach's plexus.

The second action of eserine is seen after the ganglion cells are paralysed with nicotine. It is of a simpler type, and consists of a slow and progressive increase in tone, at H, which is similar to the effect of eserine on preparations free of ganglion cells (cf. Gasser, 1926, Fig. 4). This is also the type of response

seen in certain cooled preparations, still capable of pendulum movements, but in which the ganglion cells are functionless, as shown by the absence of nicotine responses. This second effect of eserine is apparently due to the persistence of acetylcholine liberated in the genesis of pendulum movements. It is also present for about 45 sec. in Fig. 5 A, before the appearance of the peristaltic effect.

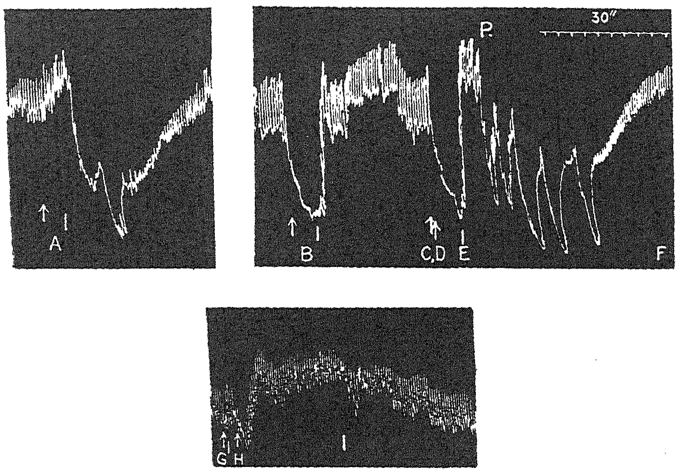

Fig. 5. Fresh rabbit's intestine ('liquid bolus') showing the effect of eserine before and after nicotine. For explanation see text. A, 1 μg. eserine sulphate for 45 sec.; B, 4 μg. $CaCl_2$ alone; C, 1 μg. eserine sulphate followed, after 10 sec., by D, 4 μg. $CaCl_2$; E, eserine and calcium washed out. After E, five peristaltic waves, starting at P. Bath fluid changed four times between E and F. Between F and G, three doses of nicotine hydrogen tartrate (10 μg.); G, fourth dose of 10 μg. nicotine hydrogen tartrate; H, 1 μg. eserine sulphate. Time in 30 sec.

Cooled intestine. In mice, the action of eserine was studied in preparations cooled for very short periods only. In such cases weak peristaltic activity may be present, or it may be in abeyance as in Fig. 6; the addition of eserine, however, brings about the prompt reappearance, at E, of large peristaltic waves of the usual type. This is a further instance of facilitation, by eserine, of latent peristalsis.

In rabbits, eserine, in doses of 1–10 μg., is ineffective in the later stages of cooling, at a time when the muscle can still respond to acetylcholine. It appears unlikely, therefore, that such doses of eserine have any action directly on the muscle fibres. This is in agreement with the findings of Anderson (1905) on the denervated pupil.

Further light is thrown on the same problem by considering the effect of eserine on the gut during electrical stimulation. This was investigated in guinea-pig preparations (Fig. 7). In order to eliminate any possible effect of

eserine in reviving peristalsis, the first preparation was cooled for 48 hr., and then examined in the usual way. At this stage, too, there is usually no response to nicotine (Vogt, 1943), indicating that the ganglion cells are inexcitable and

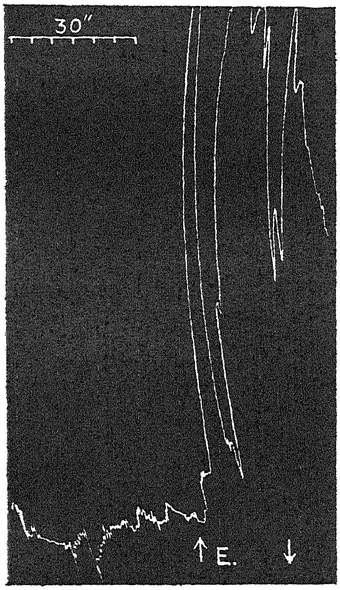

Fig. 6.

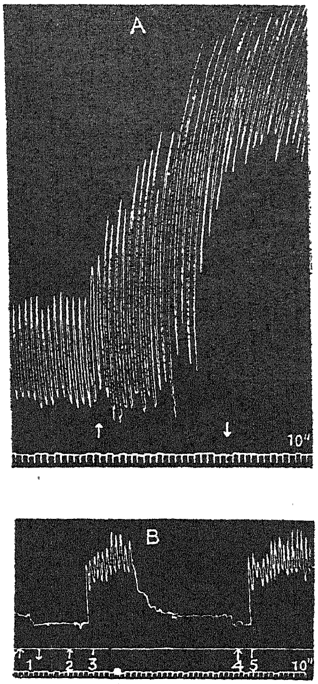

Fig. 7.

Fig. 6. Fresh mouse ileum, left in Locke's solution at 17° C. for 20 min., showing an early cooling effect. 'Liquid bolus' present, but peristalsis was at first very weak and irregular, and then absent. At E, 1 μg. eserine sulphate causing reappearance of large peristaltic contractions. Magnification × 15. Time in 30 sec.

Fig. 7. The effect of eserine on the response to electrical stimulation (54 V.) of a guinea-pig's ileum at different stages of cooling. Bath volume 10 c.c. Magnification × 10. Time in 10 sec. A. After 48 hr. cooling: eserine potentiates the effect of electrical stimulation. Shocks delivered every 10 sec. Between the arrows, 4 μg. eserine sulphate. B. After 4 days' cooling: eserine does not alter the response to electrical stimulation. Eleven shocks applied, at 10 sec. intervals, at 3, and fifteen at 5 (before and after, respectively, 10 μg. of eserine sulphate, introduced at 4). The preparation did not respond to 25 mg. KCl at 1, and to 2 mg. $BaCl_2$ at 2.

it is therefore unlikely that they participate in any response to electrical stimulation. Nevertheless, there was a clear potentiation, by eserine, of the effect of electrical stimulation (Fig. 7 A). This action of eserine was, however, absent in another preparation, from the same animal, which was cooled for 5 days (Fig. 7 B). This experiment would seem to exclude the possibility, in

the gut, of a non-specific effect of eserine on the excitability of the muscle fibres, of the type mentioned by Eccles (1937). The potentiation observed in the first preparation, cooled for 48 hr., shows that before acetylcholine synthesis is abolished, the electric current exerts its influence on the muscle partly through the 'terminal nerve reticulum' (cf. Fischer, 1944).

Potassium

It is known that potassium ions release acetylcholine from cholinergic nerve-endings (Beznák, 1934; Brown & Feldberg, 1936; Feldberg & Guimarais, 1936) and adrenaline from adrenergic fibres (Dawes, 1941). In the earthworm (Ambache *et al.* 1945), the effects of KCl were explained in terms of these two antagonistic actions on the autonomic nerves in the gut. The effect of potassium on the mammalian intestine is now considered from this point of view.

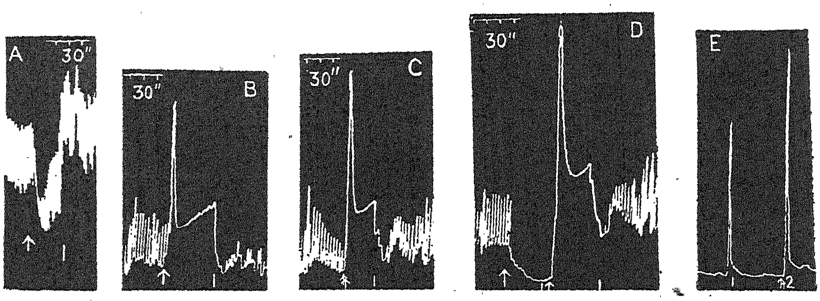

Fig. 8. Effects of potassium in fresh preparations. A–D, rabbit's intestine; lumen empty. Bath volume 5 c.c. Time in 30 sec. A. Pure inhibitory response to 1 mg. KCl. B, C, D, from another preparation; B, 6 mg. KCl alone; C, 1 μg. eserine sulphate followed, after 10 sec., by 6 mg. KCl; D, 5 mg. $CaCl_2$ at the first arrow. Washed out, and followed by 6 mg. KCl at the second arrow. E. Guinea-pig's ileum; lumen empty. Bath volume 3·5 c.c. 1, 2 mg. KCl alone; 2, 1 μg. eserine sulphate followed, 15 sec. later, by 2 mg. KCl.

Fresh intestine

Rabbits. As in the earthworm, the action of potassium is varied and complex. It produces an initial stimulation which may be followed by an inhibitory phase. Fig. 8 B shows the rapid contraction produced by a sudden rise in KCl concentration from the normal 0·02 to 0·14 %, and the inhibition which follows while the KCl is still in the bath. On washing out, the muscle at first relaxes more fully, although this may be followed, sometimes, by a partial return of contraction as the intestine resumes its rhythmic activity.

Guinea-pigs. The same effects of KCl are obtainable in guinea-pigs. Successive doses of potassium may have a diminishing effect.

Potentiation of KCl *by eserine.* Although the stimulating action of potassium is not inhibited by atropine in doses which abolish the action of acetylcholine

(cf. Vogt, 1943), yet it is enhanced by eserine, both in the rabbit (Fig. 8 B, C) and in the guinea-pig (Fig. 8 E).

Inhibitory responses to potassium. Purely inhibitory responses to small doses of KCl are sometimes seen in fresh preparations. Such an effect, on a rabbit's intestine, is shown in Fig. 8 A (dose: 1 mg. = 0·04% KCl). In the mouse, the inhibitory action of potassium, when this substance is administered in the middle of a peristaltic contraction, resembles that of adrenaline. Other instances of inhibitory responses to KCl are mentioned in the section on magnesium.

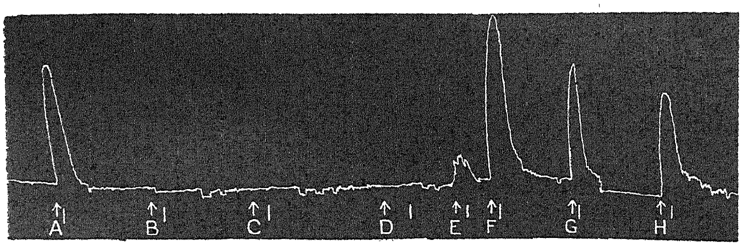

Fig. 9. Rabbit's intestine cooled for 100 hr. at 0–2° C. Magnification × 10. Note complete absence of pendulum movements; small irregularities of the base line due to bubbling. 3 c.c. bath. A, F. 10 μg. acetylcholine-HCl. B. 0·2 mg. $BaCl_2$. C. 1 mg. $BaCl_2$. D. 12 mg. KCl. E. 24 mg. KCl. G. 5 μg. acetylcholine-HCl. H. 1 μg. acetylcholine-HCl.

Cooled intestine

Rabbits. Cooling led to the eventual disappearance of both actions of potassium. Finally, the intestine failed to respond to relatively large amounts of KCl, at a time when it still reacted to small doses of acetylcholine and to electrical stimulation. Fig. 9 shows the later stages of this process, in an experiment in which the ileum was cooled for 100 hr. There was no response, at D, to 12 mg. of KCl (final concentration of KCl: 0·42% = 21 times the normal concentration of potassium in Tyrode's solution). A very small effect was seen, at E, with 0·82% KCl (= 41 times the normal). The response of the same preparation to acetylcholine is shown for comparison at A, F, G and H.

Various grades of extinction of the potassium effect were observed with shorter periods of cooling. In some cases there was no response to the first dose of 0·2% KCl, but when this was washed out a second dose produced a contraction. In more advanced cases of failure, 0·2% was ineffective (Fig. 10 F), but a slow effect was produced by 0·4% (Fig. 10 G).

Guinea-pigs. In the experiment illustrated in Fig. 7 B, KCl had no effect (at 1), although the muscle gave a good response to electrical stimulation and to acetylcholine (not shown).

Barium

Feng (1937*b*) has obtained indirect evidence that barium may produce a leakage of acetylcholine at motor nerve-endings. A similar effect on the cholinergic endings in the gut is here described.

Potentiation by eserine in the fresh intestine. The action of barium is enhanced by eserine, both in the rabbit (Fig. 11 A, B) and in the guinea-pig (Fig. 11 C).

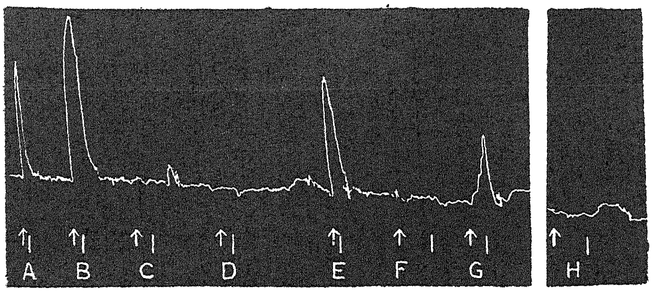

Fig. 10. Rabbit's ileum cooled for 107 hr. at 0–2° C. Magnification × 13. Bath volume 5 c.c. A. 0·5 μg. acetylcholine-HCl. B. 1 μg. acetylcholine-HCl. C, D. 8 mg. $CaCl_2$. E. 8 mg. $CaCl_2$ and 1 μg. acetylcholine-HCl. F. 8 mg. KCl. G. 16 mg. KCl. H. 100 μg. histamine acid phosphate.

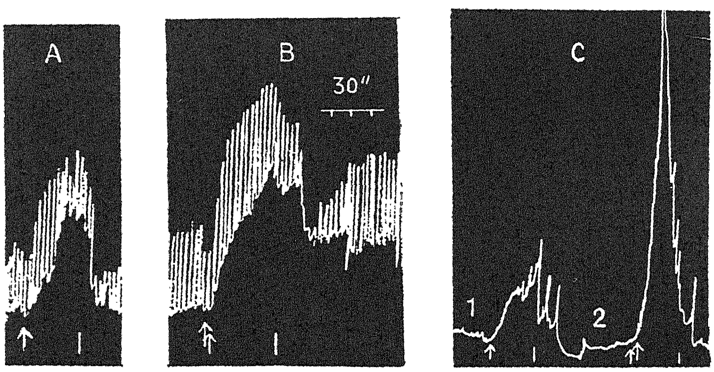

Fig. 11. Enhancement of barium by eserine in fresh preparations. Bath volume 3·5 c.c. A and B from the same piece of rabbit's intestine. Time in 30 sec. A. 0·1 mg. $BaCl_2$ alone. B. 1 μg. eserine sulphate followed, 10 sec. later, by 0·1 mg. $BaCl_2$. C, from a guinea-pig's ileum. 1, 0·1 mg. $BaCl_2$ alone; 2, 1 μg. eserine sulphate followed, after 10 sec., by 0·1 mg. $BaCl_2$.

Cooled intestine. In rabbits, cooling produced a gradual diminution in the response to barium and its eventual disappearance (Fig. 3 C). In one instance, the decrease in sensitivity to barium was noticeable after 4 hr. at 1° C. Later, delayed responses were common. Lastly, as in Fig. 9, the absence of any

response to 1 mg. of $BaCl_2$ (more than 100 times the dose to which this preparation was originally sensitive) stands in contrast to the large contractions produced by small doses of acetylcholine (e.g. 1 μg. at H). These findings were confirmed in the guinea-pig (Fig. 7 B (2)).

Calcium

According to Brown & Feldberg (1936) calcium ions prevent the release of acetylcholine, by potassium, from preganglionic nerve-endings. Recently, Feldberg & Mann (1944) have shown, also, that acetylcholine synthesis is inhibited, in cell-free extracts of brain, by an excess of calcium. In the earthworm, it was found that these properties of calcium could account entirely for its action on the gut. The action of calcium ions on the mammalian intestine has therefore been re-examined from this point of view, by investigating their effect both on rhythmic activity and on the action of other drugs.

Effect on pendulum movements.

In rabbits, the effect of calcium on the intestine depends partly upon the muscle tone. In preparations displaying little tone, $CaCl_2$ produces an immediate arrest of pendulum movements. On washing out the excess of calcium, there is usually a brief interval before rhythmic activity begins again, weakly at first but gradually returning to its previous amplitude. There is little or no change in the tone of the muscle throughout.

In other preparations in which the tone of the muscle is more marked, calcium produces, besides cessation of rhythmic activity, a prompt relaxation of the muscle (Figs. 5 B, 12 A). During the period of calcium arrest, the muscle is, however, still capable of excitation by electrical stimulation (Fig. 12 B) and by acetylcholine (Fig. 13 C).

On washing the calcium out there is, either immediately or after a short delay, a 'rebound' of the muscle over and above its original tone-level and a return of rhythmic activity. This second action of calcium is similar to an effect previously described in the crop and gizzard of the earthworm. It lasts 3–4 min., during which the muscle gradually returns to its original tone; it is enhanced by eserine (cf. Fig. 5 B, D).

Effect on peristalsis

In the mouse, as in the earthworm, peristalsis is arrested by calcium. In the one guinea-pig preparation in which peristalsis was present, this was also interrupted by calcium (Fig. 12 C). On changing the bath fluid, there was an immediate 'rebound' after-effect which lasted 3 min.

Effect on the action of other drugs

Since calcium suppresses the synthesis and release of acetylcholine, it was to be expected that it would also differentiate between drugs of the 'direct'

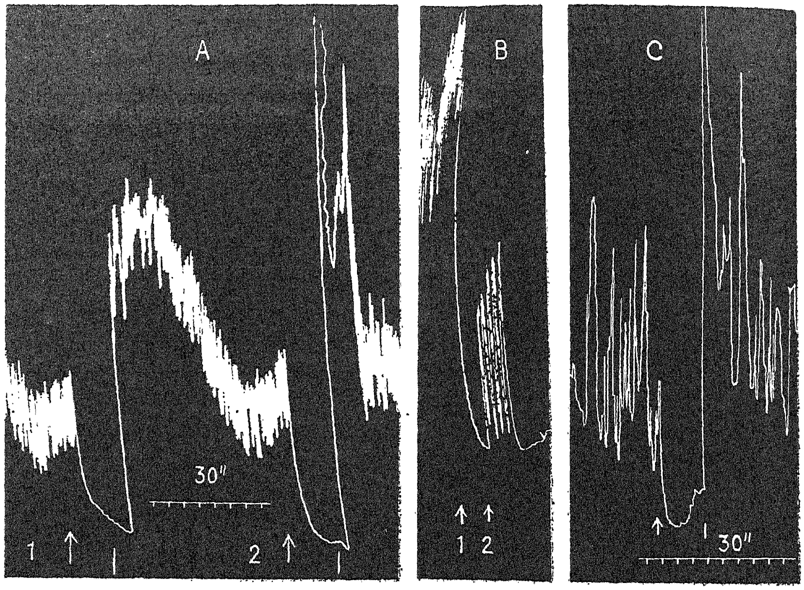

Fig. 12. Effects of calcium. A and B, rabbit's intestine (preparations with good tone). A. Bath volume 5 c.c.: 1, 4·5 mg. $CaCl_2$; 2, 9 mg. $CaCl_2$. Note the after-effect of calcium. B. Bath volume 10 c.c.: 1, 30 mg. $CaCl_2$ to the end of the tracing; 2, electrical stimulation (21 V.), five successive shocks at 10 sec. intervals. C. Guinea-pig's ileum exhibiting active peristalsis (later abolished by nicotine and by cooling for 26 hr.). Lumen slightly distended. Bath volume 10 c.c. At the arrow: 8 mg. $CaCl_2$. Note after-effect. Time for A and C in 30 sec.

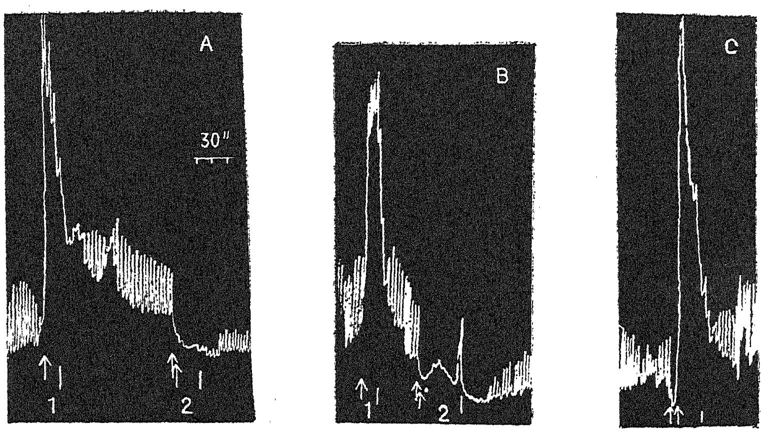

Fig. 13. Effects of calcium on the response to other drugs. Rabbit's ileum; 3·5 c.c. bath. For explanation see text. A. 1, 10 μg. nicotine hydrogen tartrate; 2, 6 mg. $CaCl_2$ followed, 10 sec. later, by 10 μg. nicotine hydrogen tartrate. B. 1, 0·3 μg. acetylcholine-HCl; 2, 5 mg. $CaCl_2$ and, after 10 sec., 0·3 μg. acetylcholine-HCl. C. 5 mg. $CaCl_2$ and, after 10 sec., 2 μg. acetylcholine-HCl. Time in 30 sec.

and 'indirect' categories. Because of the existence of two effects of calcium on the fresh preparation the interaction of calcium and other drugs was studied both during the period of calcium inhibition and during the after-effect of calcium. In the first instance, the drugs were introduced into the bath 10 sec. after the onset of calcium arrest; in the second, they were introduced immediately after the calcium was washed out.

Action of drugs during calcium inhibition. In both rabbits and guinea-pigs, the action of nicotine (Fig. 13 A), eserine (Fig. 5 C, D), potassium and barium is suppressed during this period. Acetylcholine, in doses producing the 'nicotine action' (0·1–0·5 μg.), is also ineffective (Fig. 13 B); but in doses producing the 'muscarine action' (1·5–2 μg.), it still contracts the muscle (Fig. 13 C).

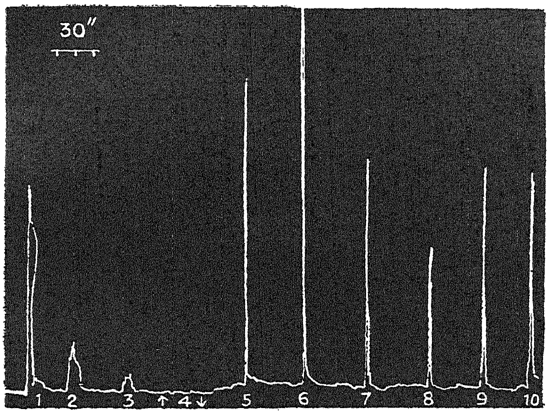

Fig. 14. Reaction of a guinea-pig's ileum to potassium during the calcium after-effect. Lumen empty; 3·5 c.c. bath. Time in 30 sec. Nos. 1–3 and 5–10: 2 mg. KCl. No. 4: 6 mg. $CaCl_2$ between the arrows.

Action of drugs during the after-effect of calcium. In rabbits, there is an increase of the effect of KCl (Fig. 8 B), of $BaCl_2$, and of acetylcholine in doses producing the 'nicotine action'.

This after-potentiation by calcium is also seen in guinea-pig preparations and is particularly striking because, in these, there is usually no change in tone, when peristalsis is absent, either in the presence of calcium or after it (Fig. 14).

It has already been mentioned that the after-effect of calcium enhances the action of eserine in facilitating peristaltic reflexes in the rabbit (Fig. 5 C–P).

It appears, therefore, that, like the rhythmic activity of the intestine, the response to drugs acting on the nerve-endings is suppressed by the presence of calcium and enhanced by its after-effect.

Effect of calcium on cooled intestine

Calcium has no effect, by itself, on cooled preparations which still react to acetylcholine (Fig. 10 C, D). Moreover, if acetylcholine is introduced into the bath after calcium, with the usual interval of 10 sec., it still contracts the muscle, although the effect may be slightly reduced. A longer soaking in calcium further reduces the effect of acetylcholine.

Magnesium

It is known that magnesium interferes with neuromuscular transmission, both in invertebrates (Pantin, 1936) and in vertebrates (various authors quoted by Sollmann, 1943, p. 877). In the gut, magnesium produces several changes attributable to a similar depressing effect on the cholinergic nerve-endings, which confirm some of the observations in the preceding sections.

In the presence of magnesium, the pendulum movements of the rabbit's intestine are diminished and preparations with good tone are relaxed (Fig. 15, 2). After the magnesium is washed out, there is no augmentor after-effect of the type seen with calcium. After repeated doses there are signs of increasing depression; pendulum movements are still present, but their amplitude is progressively reduced (Fig. 15, from 6 onwards) and later their frequency (from 10 onwards). This depression may persist for some time, despite frequent washings. The following observations suggest that it is due primarily to an action on cholinergic nerves and endings in the gut. During magnesium arrest, the muscle still responds, though less well, to electrical stimulation, and to acetylcholine in doses producing the 'muscarine action' (Fig. 15, 4). On the other hand, 'nicotine doses' of acetylcholine, and substances which affect the nerve-endings such as potassium (Fig. 15, 5) and barium, are without effect.

Reversal of the effect of potassium after magnesium. The action of various drugs was tried, as with calcium, after magnesium withdrawal. The effect of acetylcholine, barium, and histamine during this period was of the usual type, although the contraction produced showed a reduction in size corresponding to the depression of pendulum movements. The action of potassium was, however, variable. In preparations in which the original effect of potassium was purely motor, there was no change after magnesium; but in those preparations in which the initial response to KCl was complex, with a well-marked inhibitory phase, there was, after magnesium, a complete reversal of the original action of potassium. Fig. 15 illustrates such an experiment; it shows the initial response to KCl which is of the usual mixed motor and inhibitory type (Fig. 15, 1). The effect of four doses of $MgCl_2$ is then shown at 2, 3, 4 and 5. After the fourth dose of magnesium was washed out potassium was tried again; the response obtained, which was purely inhibitory, is shown at 6 and 7, and again at 13.

If the assumption is correct that the normal diphasic response to KCl is due to a stimulation of both cholinergic and adrenergic nerves in the gut, the reversal of potassium after $MgCl_2$ is explainable by a greater depression of the cholinergic fibres.

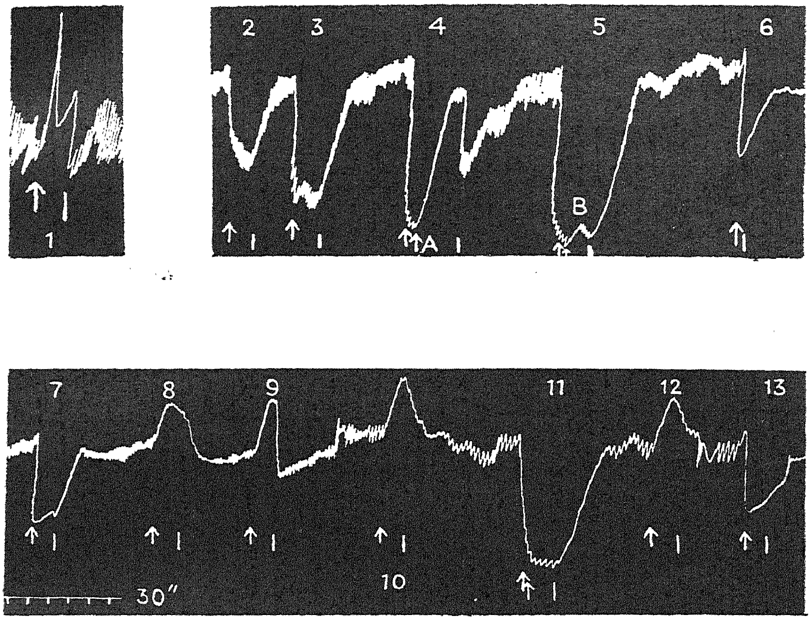

Fig. 15. Fresh rabbit's intestine. Effects of magnesium, including potassium-reversal. For explanation see text. Bath volume 5 c.c. Time in 30 sec. 1. Initial effect of 6 mg. KCl. 2. 2 mg. $MgCl_2$. 3. 3 mg. $MgCl_2$. 4. 5 mg. $MgCl_2$; at A, 1·5 μg. acetylcholine-HCl. 5. 5 mg. $MgCl_2$; at B, 6 mg. KCl. 6, 7 and 13. 6 mg. KCl. Note response to potassium now inhibitory. 8. 0·3 mg. $BaCl_2$. 9. 1·5 μg. acetylcholine-HCl. 10, 12. 30 μg. histamine acid phosphate. 11. 5 mg. $MgCl_2$ and 30 μg. histamine acid phosphate.

Histamine

The action of histamine was analysed by the methods outlined above. The results of this investigation, based on the interactions of this substance with other drugs and on the effect of cooling, would seem to place histamine in the 'indirect' category of drugs.

Guinea-pigs

Potentiation by eserine in fresh preparations. The action of histamine is enhanced by a previous dose of eserine. The effect is well marked if the usual time interval of 10 sec. is allowed between the two drugs. But in Fig. 16 C it was possible to increase this time interval to 2 min., because the effect of eserine by itself was so small. The contraction then produced by histamine was more

than double its previous height, and was followed, after the wash-out, by large rhythmic contractions, which persisted, despite several changes of bath fluid, for $4\frac{1}{2}$ min.

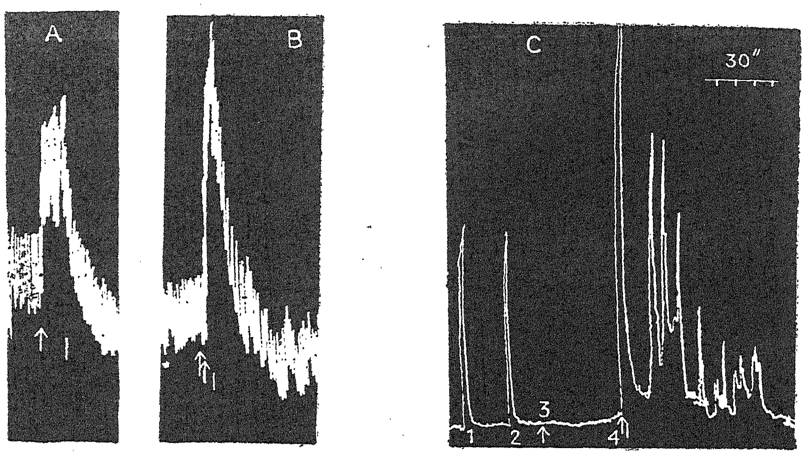

Fig. 16. Potentiation of histamine by eserine. A and B from the same piece of rabbit's intestine (5 c.c. bath). A. 5 μg. histamine acid phosphate alone. B. 1 μg. eserine sulphate and, 10 sec. later, 5 μg. histamine acid phosphate. C. From a guinea-pig's ileum; lumen empty. Bath volume 3·5 c.c.: 1, 2, 0·4 μg. histamine acid phosphate alone; 3, 1 μg. eserine sulphate followed, 2 min. later, by 4, 0·4 μg. histamine acid phosphate. Note the prolonged after-effect. Time in 30 sec.

Inhibition by calcium. Calcium abolishes the effect of relatively large doses of histamine, but very large doses of the latter are able to overcome the antagonism of calcium.

Enhancement by the after-effect of calcium. Although, in quiescent guinea-pig preparations, calcium does not affect the tone of the muscle, either during its presence in the bath or afterwards (Fig. 17, 4), yet it enhances the subsequent effect of histamine as it does that of the other 'indirect' drugs. This is illustrated in Fig. 17, where the effect of a constant dose of histamine, at intervals of 1–$1\frac{1}{2}$ min., is shown before (at 2 and 3) and after (at 5 to 9) a dose of calcium (at 4). As this was rather a large dose of calcium, the enhancement of histamine did not begin for 2 min., but it lasted much longer than the last 6 min. shown in the tracing.

Rabbits

Fresh intestine. The same effects can be demonstrated in the intestine of the rabbit, although it is less sensitive to histamine. Fig. 16 A and B shows the enhancement by eserine; care was taken to introduce the second dose of histamine, at B, well before the expected onset of any contraction due to the

eserine alone, but it was necessary to allow a short time interval for the eserine to take effect.

Calcium and magnesium abolish the action of histamine; the after-effect of calcium enhances it.

Cooled intestine. There was no response to histamine in cooled preparations. Fig. 10 shows the ineffectiveness, at H, of 100 μg. of histamine, in a preparation which still reacted well to 0·5 μg. of acetylcholine (at A) and which was originally sensitive to 5 μg. of histamine and 0·1 μg. of acetylcholine.

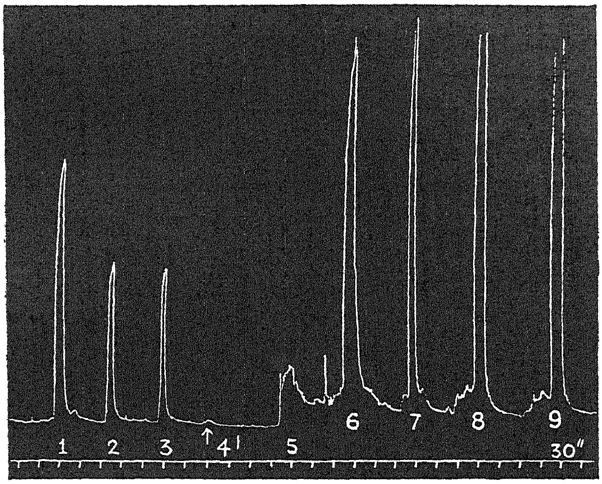

Fig. 17. Potentiation of histamine by the after-effect of calcium, in the guinea-pig's ileum. Lumen empty. 1, 0·25 μg. histamine acid phosphate; 2, 3 and 5–9, 0·2 μg. histamine acid phosphate; 4, 18 mg. $CaCl_2$ between the arrow and the vertical line. Time in 30 sec.

The release of acetylcholine by drugs of the indirect category

In the preceding section the evidence that the 'indirectly' acting drugs release acetylcholine has been by inference, with the exception of KCl, the ability of which to liberate acetylcholine is well established. In this section, evidence of a more direct kind is adduced to show that such a release also occurs with histamine and barium.

Histamine

A liberation of acetylcholine from the intestine by histamine can be demonstrated by both methods of incubation described. In the first there was, with histamine, a distinct increase in the amount of acetylcholine liberated by 10 g. of guinea-pig's ileum into the surrounding bath fluid. The acetylcholine activity of the sample of bath fluid collected after an initial 'resting' period of 10 min. was, in two different experiments, approximately 0·1 μg./c.c. During the next 10 min. period, 2 μg. of histamine were added to the bath

every third minute, making a total of 6 μg. The activity of the sample collected at the end of this 10 min. period was, in both experiments, approximately 0·5 μg./c.c.

In the second method, the total amount of acetylcholine in the tissue and in the bath fluid was determined. Accurate assays were possible because of the very much larger amounts of acetylcholine present in the extracts. Two sets of strips were incubated simultaneously for 40 min.; one received 1 μg. of histamine in 0·1 c.c. of distilled water at 5 min. intervals, up to a total of 6 μg. The control strips received 0·6 c.c. of distilled water after incubation. The acetylcholine contents of the two extracts were:

Control strips	6·25 μg./g.
Strips incubated with histamine	9·6 μg./g.

showing a liberation of 3·35 μg./g. during the 40 min. The activity of both extracts was completely destroyed by boiling for 1 min. with 1/10th their volume of N/3 NaOH. It was not due to histamine because the frog's rectus abdominis is insensitive to this substance (Chang & Gaddum, 1933). In another experiment a total of 1·65 μg. of histamine liberated in 30 min. 1·7 μg./g. of acetylcholine.

Barium

In this case, only the second method of incubation was used. 0·05 c.c. of 1% $BaCl_2, 2H_2O$ was added every 10 min. to the 'active' strips of intestine, making a total of 0·2 c.c. (2 mg. of $BaCl_2, 2H_2O$). The control pieces were incubated without barium, but in order to treat the two samples identically, 0·2 c.c. of 1% $BaCl_2, 2H_2O$ was added to the control sample after it was boiled. The two samples then received 0·1 c.c. of 1% Na_2SO_4 each, which amount, it was calculated from the solubility product of $BaSO_4$, would precipitate very nearly all the barium present. The results of the assay showed a great difference between the two samples; the values obtained were:

Control strips	5·3 μg./g.
Strips incubated with barium	11·8 μg./g.

showing a liberation of 6·5 μg./g. during 40 min. In another experiment, 4 mg. $BaCl_2, H_2O$ liberated 3·9 μg./g. in 1 hr. The activity of the control and 'active' samples was destroyed by alkali at room temperature.

As a further control, a sample consisting of reagents only showed no activity when tested on a frog's rectus.

DISCUSSION

As a result of the present experiments and of the previous work on the earthworm, it is suggested that the drugs which contract the muscle indirectly, i.e. by releasing acetylcholine, can be distinguished from those which affect the muscle directly by the following criteria: (*a*) ineffectiveness after cooling,

at a time when the muscle fibres still retain their excitability to acetylcholine and to electrical stimulation, (*b*) potentiation by eserine, (*c*) inhibition by an excess of calcium (or by magnesium), (*d*) potentiation during the calcium after-effect. In the present experiments, too much importance is not attached to any single one of these criteria; but, when taken together, the evidence provided by them all points in the same direction. Their value is confirmed, in the case of barium and of histamine, by demonstrating a definite release of acetylcholine.

Beyond eliminating, with nicotine, those drugs which act on the ganglion cells, it is as yet impossible to narrow down further the site of action of any augmentor substance obeying these criteria. In each case, there are two further possible sites of action: (1) the parasympathetic post-ganglionic nerve fibres and their endings, (2) the nerve net or 'terminal reticulum', the importance of which has recently been discussed by Fischer (1944). In the gut this consists of a syncytium of 'interstitial nerve cells' interconnected by very fine fibres. These cells were first described by Cajal (1893), and attention has already been drawn, by van Esveld (1928) and by Boeke (1940), to the possible role they may play in initiating pendulum movements. The effect of cooling on the rhythmic activity of the intestine, and of eserine in preparations in which the ganglion cells are non-functional is indeed suggestive of the existence, in the gut, of some other cholinergic structure apart from the pre- and post-ganglionic elements of the parasympathetic system.

With regard to the disappearance of pendulum movements, my results appear to disagree with those of Gunn & Underhill (1914), who reported the presence of very weak pendulum movements in preparations cooled for 118 hr. In their experiments, however, the cooling was less severe (3–7° C.) than in mine (0–2° C.). The bearing of these results on the nature of pendulum movements will be discussed in a later paper; but it is interesting to note that potassium and barium are able to release stored acetylcholine, and so contract the muscle, for some time after transmission has failed in the nervous mechanism both for peristalsis and, possibly, for pendulum movements.

Calcium. Since the muscle-fibres still respond, during calcium inhibition, to electrical stimulation and to acetylcholine, it would appear that the prompt arrest of pendulum movements in the rabbit, and of peristalsis in the earthworm and in the mouse, is effected by 'cutting off' the acetylcholine concerned in the transmission of these processes; the inhibition of indirect drugs would be due to the same effect. This view disagrees with that taken by Feng (1937*a*) and adopted by Cowan (1940). The former observed phenomena similar to these in skeletal muscle, and attributed the inhibition produced by calcium to a curarizing effect arising from an excessive release of acetylcholine at the neuromuscular junction. This deduction was not confirmed by direct experiment; it is in fact diametrically opposite to the results of Brown & Feldberg

(1936), who, in perfusion experiments on the superior cervical ganglion, showed that calcium (*a*) does not release acetylcholine by itself, and (*b*) prevents the release of acetylcholine by potassium ions.

The after-effect of calcium is similar to that reported by Feng & Shen (1937). Since it is enhanced by eserine and disappears on cooling, it appears to be associated with an increased liberation of acetylcholine at the nerve-endings, either during peristalsis or pendulum movements, or in response to the indirectly acting drugs.

Potassium. As in the earthworm the various effects of potassium disappear on cooling. They can be interpreted in terms of stimulation of the cholinergic and adrenergic fibres in the gut. The response obtained in any given preparation will be the resultant of these two antagonistic actions. The absence of any residual effect of KCl on the muscle fibres after cooling is in agreement with Buchtal & Lindhard's observation (1939) that KCl has no action on the fibre substance in skeletal muscle.

The initial stimulation produced by potassium obeys the four criteria enumerated above; the conclusion that this action is due to a release of acetylcholine (Ambache *et al.* 1945) is strengthened by the present experiments. The inhibitory action which follows the first suggests a stimulation of adrenergic fibres.

Barium. The concentration of barium used in these experiments did not exceed 1 in 3000. Such doses do not appear to affect the muscle fibres directly and obey the four criteria adopted above. Moreover, the suggestion made by Feng (1937*b*) that barium induces leakage of acetylcholine receives direct confirmation in the incubation experiments.

SUMMARY

1. The effect of abolishing acetylcholine synthesis in mammalian intestines (by cooling) has been investigated. The earliest change seen in cooled preparations is a loss of peristalsis. It occurs before the disappearance of nicotine responses and is attributable to failure of transmission in Auerbach's plexus. At first, this effect is only partial: peristalsis is latent, but can be facilitated by eserine. Later, the loss is permanent.

2. After 4 or 5 days' cooling, pendulum movements are abolished; a smooth muscle preparation is obtained which, although it does not exhibit rhythmic activity, still responds to electrical stimulation and to acetylcholine, but not to KCl, $BaCl_2$, or histamine

3. Cooling abolishes the response to eserine (1–10 μg.). In fresh preparations, eserine has a twofold action, depending on the preservation of acetylcholine from two different sources in the gut.

4. Potassium ions first contract and then inhibit the fresh gut. The two effects are abolished by cooling. The first is enhanced by eserine and

appears to be due to a release of acetylcholine from the cholinergic nerve-endings in the gut. After magnesium, the action of KCl is predominantly inhibitory; it is suggested that this action is due to a stimulation of adrenergic fibres.

5. Barium and histamine act by releasing acetylcholine. Such a release is demonstrated in surviving strips of gut.

6. Calcium ions inhibit the spontaneous activity of the gut and the effect of all drugs which act indirectly, i.e. by releasing acetylcholine, but not electrical stimulation or doses of acetylcholine which act directly on the muscle. It is suggested that these actions of calcium are due to inhibition of acetylcholine synthesis and release, at the cholinergic nerve-endings. A 'rebound' type of after-effect is described which appears to be nervous in origin.

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THE METABOLISM OF KIDNEY SLICES FROM NEW-BORN AND FULL-GROWN RATS

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In the last few years the infantile human kidney has been shown to function in many respects rather differently from the adult organ. Thus, in the infant, the glomerular filtration rate seems to vary with the hydration of the body, and, on the basis of surface area, the glomerular filtration rates and also the urea clearances are lower (Barnett, 1940; Barnett, Perley & McGinnis, 1942; McCance & Young, 1941; Young & McCance, 1942). There is a structural basis for some of these functional differences, for Clara (1936) and Grunewald & Popper (1940) have shown that the glomerular tufts at birth are covered with thick columnar or cubical cells through which filtration must be relatively slow. These cells were described and pictured at least 72 years ago in an English text-book of histology (Klein, Burdon-Sanderson, Foster & Brunton, 1873), so that they are no new discovery (McCance, 1943), but it has taken physiologists a long time to appreciate their importance. Furthermore, the Na, K and Cl clearances are lower at birth than the corresponding clearances in later life, and this suggests tubular differences, a view which is supported by studies of the osmotic pressure of the urine in infancy and also by observations on the response to posterior pituitary extract (Heller, 1944). In order to investigate some of these aspects of developmental physiology from another angle it was decided to compare the O_2 uptake and the metabolism of the kidneys in new-born and mature animals by means of tissue slices. The experiments described in this paper have been carried out on rats, but kidneys from cats, pigs and human beings are also being investigated.

METHOD

Technique

The adult animals were killed by severing the carotid artery. The new-born animals, which were always used within 24 hr. of birth, were killed by decapitation. The kidneys were removed immediately and transferred to a dish containing a Ringer-phosphate solution. This was a modification of that used by Krebs (1933) and was made up in the following way: To 103 vol. of 0·9% NaCl were added 4 vol. of 1·15% KCl, 1 vol. of 3·82% $MgSO_4 . 7H_2O$, 1 vol. of 2·11% KH_2PO_4

and 21 vol. of phosphate buffer, pH 7·4, prepared by taking 40 c.c. 0·025 M-Na_2HPO_4 and 0·5 c.c. 2 N-HCl and diluting to 100 c.c. Slices of kidney, approximately 0·3 mm. in thickness, were cut, trimmed as quickly as possible and transferred to the metabolism bottles of a Barcroft apparatus. The total volume of fluid in the outer compartment was 2·7 c.c., the basis of which was Ringer phosphate. The inner compartment contained 0·3 c.c. of 20% NaOH and a small roll of Whatman no. 40 filter paper (Dixon, 1943). The bottles were then attached to the manometers, the whole apparatus evacuated and then filled with O_2 before being transferred to a water bath at 38° C. (Dixon & Tunnicliffe, 1923). The bottles moved to and fro 125 times per min., and after 5 min. for equilibration, the taps were closed and the first reading taken. This procedure was followed in each experiment and the interval between the death of the animal and the first reading was always about 40 ($\pm$5) min.

The O_2 uptakes have been measured at 15 min. intervals for an hour, and a further reading taken after 2 hr., (*a*) without any additions at pH 7·4 and 6·8, (*b*) after adding the following hormones at pH 6·8 and 7·4: 0·3–0·9 unit of posterior pituitary extract ('Pitoxylin', manufactured by Messrs Oxo) and 0·05–0·5 mg. desoxycorticosterone phosphate (presented by Messrs Organon Ltd.), (*c*) in the presence of the following substrates at pH 7·4: 0·02 M-Na lactate, 0·02 M-Na succinate and 0·02 M-*dl*-methionine. R.Q. determinations were made by the Dixon-Keilin method (Dixon, 1943) and all estimations of N were carried out by the micro-Kjeldahl technique. All figures given in this paper are the average results of 6–10 experiments. Owing to the inherent variability of excised tissues, the control for each experimental substrate has always been set up from the kidneys of the same animal (or, if newly born, from the kidneys of litter mates). Consequently, figures given for the control experiments in Tables 2–5 are not always the same.

Expression of results

In work with tissue slices the respiration rate is usually denoted by the symbol Q_{O_2} and is defined as the c.mm. of oxygen (at N.T.P.) taken up per hr. per mg. dry weight of tissue. This is an unsatisfactory method for two reasons. First, the rate at which O_2 is taken up by kidney slices usually falls off with the passage of time so that measurement over the first 15 min. or 30 min. gives a result very different from the one given by measurement over the first hour or over the two hours together. In these experiments, readings have been taken every 15 min. at least for the first hour, and the rate over each interval of time expressed as the c.mm. of O_2 taken up per min. In the second place the slices tend to lose weight as they are shaken (Bach, 1944) and, in consequence, the results obtained vary with the time at which shaking has been stopped and the slices removed for drying and weighing. Some workers have preferred to express their O_2 uptakes per mg. initial wet weight (Bach, 1944; Elliott, Grieg & Benoy, 1937; Field, Belding & Martin, 1931; and others). This is a great improvement in principle but it is not an easy matter to weigh the freshly cut slices accurately, and so, in order to avoid both these difficulties, the present O_2 uptakes have been expressed per mg. of N in the freshly cut slices. This figure was obtained in the following way: At the end of the experiment the N was determined both in the slices and in the fluid in which they had been respiring. The analyses were made separately, and thus the total N originally present in the slices and the percentage of N which had diffused out of them during the experiment could be ascertained.

Table 1 shows the results expressed both per mg. final dry weight and per mg. total N in the slices taken for metabolism. Each comparison was made on kidneys from the same rat. The figures in the 1st and 2nd columns represent the O_2 uptakes per mg. of the final dry weight over the first 30 min. The figures

TABLE 1. A comparison of the O_2 uptakes of rat kidney slices expressed as c.mm./mg. final dry weight—or as c.mm./mg. N in the original slices. The figures represent the O_2 uptake during the first 30 min. of experiment

	c.mm./mg. final dry weight		c.mm./mg. total N			
	Slice weighed after 30 min.	Slice weighed after 120 min.	N determined after 30 min.	N determined after 120 min.	% loss of N in 30 min.	% loss of N in 120 min.
	11·6	16·9	81·5	82·5	18·9	38·6
	12·4	15·3	82·0	82·5	23·4	37·4
	12·3	14·1	81·0	77·5	19·3	34·4
	10·1	14·2	78·5	83·5	15·0	32·5
	11·5	17·0	80·0	90·0	13·4	35·4
	9·3	10·7	67·0	65·0	14·9	28·0
	11·2	13·3	82·0	81·5	17·8	29·8
	9·7	14·1	71·0	69·5	16·9	43·0
Av.	11·0	14·5	77·9	79·0	17·5	34·9

in the 1st column were obtained by removing the slices and drying them at the end of 30 min., and those in the 2nd column by removing them after they had been shaken for 2 hr. The figures in the 2nd column are higher because the slices from which these figures were derived had been allowed a further $1\frac{1}{2}$ hr. in which to lose weight, and consequently their dry weights were proportionally lower than those of the slices which had only been shaken for 30 min. This is proved by columns 3 and 4, in which the same O_2 uptakes are expressed per mg. N in the tissues taken for metabolism. It will be seen that on this basis the fictitious differences have disappeared. Columns 5 and 6 show the amount of N that the tissues lost after being shaken for 30 and 120 min. respectively.

Stating the O_2 uptakes per mg. of N in the fresh slices has its advantages also when the organs of new-born animals are being compared with those of adults, for the tissues are more hydrated at birth than at maturity (Shohl, 1939). Indeed, the kidney of the new-born rat may contain only some 15–16 % of dry matter, whereas that of an older animal as much as 25 %. Hence fresh weight would be an unsatisfactory basis on which to compare these organs. It would, however, if used have the effect of lowering all the O_2 uptakes for the new-born tissues given in this paper.

RESULTS

Kidney slices

O_2 *uptake in Ringer phosphate without added substrate.* Fig. 1 shows the rates of O_2 uptake at pH 7·4 by slices of the kidney from mature and new-born rats. It will be noted that during the first 15 min. the adult tissues took up O_2 at

the rate of 3·0 c.mm./min. and those from the newly born animals at the lower rate of 2·0 c.mm./min. With the passage of time, however, the O_2 uptake of the adult kidneys fell off rapidly while that of the new-born did not, so that at the end of 1 hr. the uptakes of both were almost the same, and during the second hour the slices from the new-born rats took up O_2 more rapidly than those from the full-grown animals. Slices from rats aged 3 weeks showed characteristics both of the adult and new-born organs. Their O_2 uptakes

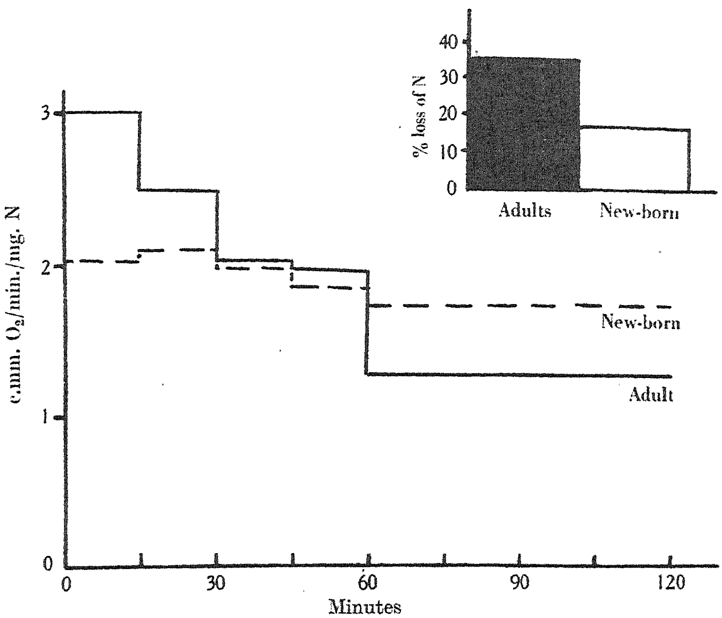

Fig. 1. The uptake of oxygen and loss of nitrogen by slices of rats' kidneys at *p*H 7·4.

averaged 3·2 c.mm./min. in the first 15 min., a little higher than those of the full-grown animals, but they fell off very slowly, a characteristic of the new-born, and were still 2·85 c.mm./min. in the last hour.

The percentage of N diffusing out of the slices taken from fully grown rats was large. In the experiments shown in Fig. 1 the average loss over a period of 2 hr. was 35 %, but in other grouped experiments losses have averaged as much as 49 %. Much less N came away from the kidneys of young animals. Slices from rats aged 3 weeks lost 27 % of their N during the 2 hr. experiments, and those from new-born rats only 16·4 %. Thus the amount of N which the slices lost depended very much upon the age of the animal, but the nature of the N did not, for at all ages 60 % was in the form of protein (i.e. was precipitated by trichloroacetic acid), and there was practically no free NH_3 in the non-protein fraction.

Effect of pH. The experiments just described were carried out at pH 7·4. When the pH was lowered to 6·8, less N came away from the tissues of mature rats (Table 2). The O_2 uptake was little affected by this change for the first ½ hr. but it fell off less rapidly in the last 1½ hr. so that the final O_2 uptake was a little higher. Lowering the pH from 7·4 to 6·8 slightly reduced the O_2 uptake of slices from new-born rats (Table 2). It did so equally over the first 15 min. and during the last hour. It had, however, no significant effect upon the loss of N.

TABLE 2. Effect of pH on the O_2 uptake and loss of N

		Rate of O_2 uptake, c.mm./min./mg. N					
Age	pH	0–15 min.	15–30 min.	30–45 min.	45–60 min.	60–120 min.	% loss of N
Mature rats	7·4	2·70	1·95	1·55	1·31	1·00	48·9
	6·8	2·75	2·25	1·85	1·73	1·50	35·8
New-born rats	7·4	2·3	2·11	1·94	1·99	1·85	16·4
	6·8	2·04	1·97	1·84	1·84	1·51	17·3

Posterior pituitary extract. 0·3, 0·6 and 0·9 unit of posterior pituitary extract (Pitoxylin) were added to the Ringer phosphate solution in which the tissues were respiring. 0·9 unit appeared to be the most effective. This dose of Pitoxylin was investigated at pH 6·8 and 7·4, and may be said to have had three distinct but probably related effects upon the metabolism of the tissues from fully grown animals (Table 3). First, the O_2 uptake was considerably raised at both pH's, the initial level being slightly higher at pH 7·4. Secondly, at pH 6·8 the O_2 uptake fell off very slowly and finished considerably higher than it did at pH 7·4, or when no Pitoxylin had been added. In this the posterior pituitary extract appeared to enhance the effect of pH alone. Thirdly, Pitoxylin prevented the cells losing N. This may have been the essential feature of its action, and it certainly offers some explanation of the first two effects.

TABLE 3. The effect of 0·9 unit Pitoxylin on the O_2 uptake and loss of N

		Rate of O_2 uptake, c.mm./min./mg. N					
Age	Additions and pH	0–15 min.	15–30 min.	30–45 min.	45–60 min.	60–120 min.	% loss of N
Mature rats	None at 7·4	2·70	1·95	1·55	1·31	1·00	48·9
	Pitoxylin at 7·4	4·15	3·54	3·45	2·86	2·26	38·9
	None at 6·8	2·75	2·25	1·85	1·73	1·50	35·8
	Pitoxylin at 6·8	3·87	3·70	3·62	3·44	3·30	23·4
New-born rats	None at 7·4	2·31	2·39	2·08	2·11	1·82	17·5
	Pitoxylin at 7·4	2·98	2·40	2·29	2·27	2·05	16·5
	None at 6·8	2·04	1·97	1·84	1·84	1·51	17·3
	Pitoxylin at 6·8	2·24	2·05	2·08	1·94	1·94	13·1

Pitoxylin slightly raised the O_2 uptake of slices from the kidneys of the new-born rats during the first 15 min. of the experiment when the pH was 7·4, but after that time the rate of respiration was very little greater than that of

the control slices to which no hormone had been added. At pH 6·8 Pitoxylin had similar effects, but there was a smaller increase during the first 15 min., and a slightly larger one during the second hour. When Pitoxylin was added to the slices from new-born animals, it did little to reduce their small losses of N. A small effect, however, was observed at pH 6·8.

Some of these effects on the adult tissues were undoubtedly due to the acetic acid which is present in commercial extracts of posterior pituitary. The *British Pharmacopoeia* specifies that sufficient acetic acid should be added to bring the extracting fluid to pH 3·0, and a concentration of 0·0038 M is sufficient to effect this. Such a concentration of acetic acid appreciably raised the O_2 uptake of the slices (Table 4) but did not raise it so high as Pitoxylin,

TABLE 4. Effect of Pitoxylin, acetic acid, extract of dried pituitary, etc. upon the tissues of mature rats at pH 6·8

	Rate of O_2 uptake, c.mm./min./mg. N					
Additions	0–15 min.	15–30 min.	30–45 min.	45–60 min.	60–120 min.	% loss of N
None	3·0	2·34	2·32	2·16	1·68	32·2
Commercial extract (Pitoxylin)	4·21	3·69	3·58	3·62	3·25	24·5
Acetic acid	3·52	2·94	2·91	2·96	2·41	31·4
Extract of dried gland	4·0	3·8	3·8	3·06	2·85	23·1
Extract of dried gland + acetic acid	4·15	3·90	4·04	4·07	3·78	21·6

nor did it prevent the slices from losing N. In the hope of disentangling some of these effects, acetone-dried posterior pituitary lobe was obtained. 0·3 g. of this material was well shaken with 9 c.c. 0·9% NaCl and centrifuged. 0·3 c.c. of the supernatant fluid was added to 2·4 c.c. of the Ringer phosphate solution in which the tissues were to respire. Respiration was measured in the presence of this extract of posterior pituitary lobe both with and without the addition of 0·0038 M-acetic acid and the results are shown in Table 4. The extract alone raised the O_2 uptake, but the combination of acetic acid and the saline extract not only raised the O_2 uptake slightly more than the posterior pituitary alone, but succeeded in maintaining it at the raised level. This double effect is characteristic of the commercial extract, Pitoxylin, at pH 6·8 (Table 3). All posterior pituitary extracts seemed to decrease the loss of N by the slices.

The oxytoxic and pressor activities of a posterior pituitary extract are destroyed by adding an equal volume of 2 N-NaOH to it, allowing to stand for 1 hr. and then neutralizing before use, but this treatment when applied to Pitoxylin did not destroy the factor, or factors, affecting the metabolism of the kidney slices. It is concluded, therefore, that the respiratory effects were brought about by some unrecognized substances in the extracts. It is possible that the effects were due simply to the addition of respirable nitrogenous material, but if so 0·9 unit of Pitoxylin, which contained only 0·06–0·07 mg. N, must have provided enough to saturate the enzyme systems, for the saline extracts contained from 0·25 to 0·37 mg. N, and their effects were very much the same.

Desoxycorticosterone phosphate. The best known salt of desoxycorticosterone is the acetate, but this is practically insoluble in water and was, therefore, unsuitable for the purpose in hand. The phosphate, however, is soluble and its effects were investigated at two concentrations and at pH 6·8 and 7·4. When 0·5 mg. of the hormone was added to the Ringer phosphate solution it was found always to lower the O_2 uptake of the adult tissues, slightly at pH 6·8 and very considerably at pH 7·4 (Fig. 2). In this connexion it is interesting

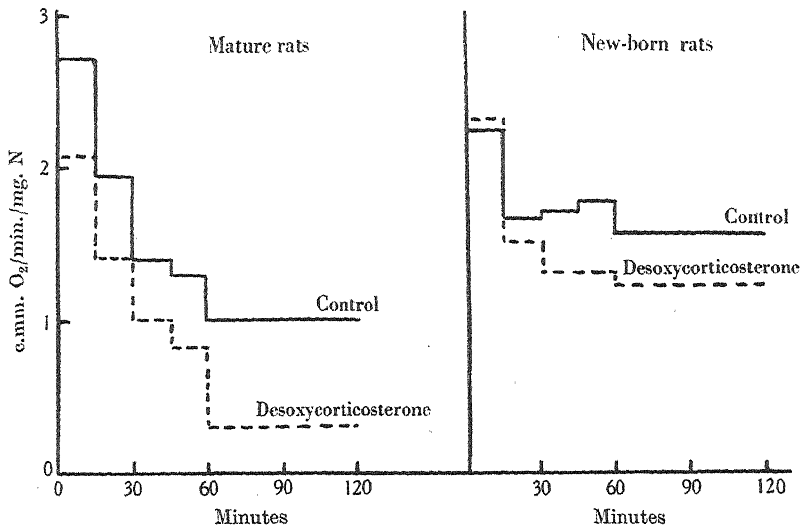

Fig. 2. The effect of 0·5 mg. of desoxycorticosterone phosphate on the uptake of oxygen by kidney slices at pH 7·4.

to note the toxicity of large doses upon the kidney of the intact animal (Selye, Hall & Rowley, 1945). 0·5 mg. slightly lowered the respiration rate of slices from new-born rats but its effect was less than in later life. Whatever the age of the animals 0·05 mg. did not alter the O_2 uptake at either pH. This hormone had little influence on the loss of N by kidney slices though at all ages it tended to decrease it at pH 6·8 when the dose was 0·5 mg.

Succinate, lactate and dl-*methionine.* The effect of adding 0·02 M concentrations of these compounds to the Ringer phosphate solution in which the slices from adult animals were respiring is shown in Table 5. These experiments have only been carried out at pH 7·4, and except in the case of lactate, at only one concentration. Doubling the dose of lactate had no greater effect. All these substrates raised the O_2 uptake considerably. Their effects, however, were rather different. Lactate raised it in the first 15 min. only from 3·0 to 4·0 c.mm./min., but the rate was still above 3 in the second hour, whereas the uptake in Ringer phosphate alone had fallen to 1·27. Succinate and methionine raised the initial rate to about 5·2 and 4·3 c.mm./min. respectively, but they

TABLE 5. The effect of lactate, succinate and *dl*-methionine at *p*H 7·4

		Rate of O_2 uptake, c.mm./min./mg. N					
Age	Substrate	0–15 min.	15–30 min.	30–45 min.	45–60 min.	60–120 min.	% loss of N
Mature rats	None	3·01	2·48	2·02	1·97	1·27	35·3
	0·02 M-lactate	4·02	3·59	3·73	3·34	3·32	32·0
	0·02 M-succinate	5·16	4·55	3·74	3·60	2·76	37·2
	0·02 M-*dl*-methionine	4·3	3·05	2·66	1·99	1·24	38·7
New-born rats	None	2·03	2·12	1·99	1·85	1·72	17·6
	0·02 M-lactate	2·72	2·58	2·58	2·64	2·44	18·7
	0·02 M-succinate	3·40	3·00	2·94	2·79	2·53	22·0
	0·02 M-*dl*-methionine	2·11	2·18	2·05	2·03	1·86	18·4

did not maintain it nearly so well as lactate. The O_2 consumption with succinate fell to 2·5 c.mm./min. in the second hour, but with methionine it fell to 1·24, the same as that of the control. Table 5 also shows that these 3 compounds did little to raise the O_2 uptakes of the tissues of new-born animals. If each result is considered as a percentage of the control value, the initial effects of succinate and lactate were not so different from those found for the mature animals, but, however the data be expressed, the influence of the substrates during the 2nd hour was very much less. The uptake in the presence of lactate fell off very little, if at all, and the uptake in succinate was passably well maintained, but the uptake of the controls also remained relatively steady. The effect of methionine was trifling throughout. The substrates did not appreciably alter the losses of N either at birth or maturity.

Liver slices from adult and new-born rats

The O_2 uptake of liver slices taken from mature and new-born animals has also been studied. In Ringer phosphate solutions at *p*H 7·4 the rate at which mature liver tissue took up O_2 did not fall off as it did so characteristically with the kidney (Table 6). Furthermore, the O_2 uptake of the tissues from the new-

TABLE 6. Rate of O_2 uptake by liver tissue, c.mm./min./mg. N

Age	Substrate	0–15 min.	15–30 min.	30–45 min.	45–60 min.	60–120 min.	% loss of N
Adult rats	None	1·52	1·31	1·31	1·33	1·21	29·5
	Pitoxylin, 0·9 unit	1·75	1·41	1·31	1·33	1·20	27·3
New-born rats	None	1·78	1·73	1·75	1·73	1·46	32·0

born rats was as high or higher throughout the whole period of observation. Posterior pituitary extract has also been added to the liver slices taken from adult animals—largely to control the observations made on the kidney. The effects were negligible. The loss of N from the liver slices was not so great as it was from adult renal tissues; it was the same at birth as in adult life, and was unaffected by pituitary extracts.

DISCUSSION

These experiments have demonstrated that the kidney slices of a new-born animal may behave very differently from those of an adult of the same species. These differences are certainly complicated and they may be interrelated. Considered as a whole the metabolism of the new-born kidney seems to be characterized by a stability and independence which are absent from that of the adult organ. Thus the O_2 uptake is well maintained without the addition of substrates, responds poorly or not at all to substances which raise the uptake of the adult organ, and resists agents which depress the adult rate. In fact, most of the present results could be explained by supposing that the new-born cells survived removal, section and shaking in Ringer phosphate solution much better than the adult tissues. How far some of these differences can be related to the fact that the new-born tissues lose less N to the surrounding medium it is not possible to say. It is, however, certain (from the examination of the sections) that the adult tissues undergo profound histological disorganization during the two hours in the respiration chamber, whereas the new-born tissues remain relatively normal.

It is generally recognized (Needham, 1931; Lightbody, 1938) that the enzymes characteristic of the adult state appear at different ages, and develop at different rates: the small response of the new-born tissues to added substrates suggests that their specific enzyme systems are not yet fully developed at birth. It seems probable, however, that the respiration of the kidney slices from young and old rats follows the same final path, for 0·001 M-CN prevented any O_2 being taken up at either age. Furthermore, the R.Q. of the tissues at birth, determined over a 30 min. period, averaged 0·913 (0·88 and 0·95) and at maturity 0·920 (0·90 and 0·94), which indicates that similar materials were being oxidized at both ages. At neither age was there any aerobic glycolysis. Nevertheless, young animals may have a source of energy other than the usual aerobic processes. Anaerobic glycolysis by the rat's kidney has been shown to be greatest before birth and is still active at birth although negligible later in life (Needham, 1931). The work of Himwich and others (Fazekas, Alexander & Himwich, 1941; Himwich, Baker & Fazekas, 1939; Himwich, Bernstein, Herrlich, Chester & Fazekas, 1942; Himwich, Fazekas & Alexander, 1941) has shown that the prolonged survival time of infant rats in an atmosphere of N is due to their low cerebral metabolism and to some anaerobic source of energy.

It has previously been considered that the O_2 consumption is higher the younger the animal (Davis, 1937; Schuler, 1943; Pearce, 1936), but these authors did not measure the metabolism of rats (or mice) under the age of 4 weeks. Tyler & van Harreveld (1942), however, measured the respiration of the developing rat's brain, and showed that the O_2 uptake was lowest during the first 7 days of life, after which it rose, and reached a maximum between

the 4th and 7th week. Thereafter it gradually fell off, but always remained considerably higher than it had been at birth and stabilized itself at the adult level at about the 20th week of life. These results are to be compared with the present findings that over the first 15 min. in the manometer the O_2 uptake by kidney slices is lowest at birth, but may be as high or higher at the age of 3 weeks than at maturity.

Summary

1. A study of tissue slice technique has shown that it is better to express results per mg. total N in the fresh slices rather than per mg. final dry weight.

2. A comparison of the O_2 taken up in Ringer phosphate solutions by kidney slices from new-born and mature rats has shown that these tissues differ in the following ways:

(*a*) At maturity, the O_2 uptake begins at about 3 c.mm./min./mg. N but soon declines, and the slices lose from 40–50% of their N in 2 hr. A change in *p*H from 7·4 to 6·8 has little effect on the O_2 uptake, but decreases the amount of N lost. At birth, the O_2 uptake is initially less rapid, but this lower rate is well maintained for 2 hr., and the loss of N is only some 17%. The uptakes and losses of N are almost unaffected by a change in *p*H from 7·4 to 6·8.

(*b*) Posterior pituitary extracts diminish the loss of N and raise the O_2 uptake, and large doses of desoxycorticosterone phosphate depress the O_2 uptake of mature tissues. These additions make little difference to the renal tissues of newly born rats. Possible reasons for these and other effects of these substances have been discussed.

(*c*) The addition of various substrates such as 0·02 M-lactate, -*dl*-methionine, or -succinate raises the O_2 consumption of kidney slices from mature rats, but has less effect on those from new-born rats.

3. Slices from the livers of new-born rats took up as much or more O_2 as those from the livers of mature animals over the whole period of observation, and lost about the same percentage of their N. Posterior pituitary extracts had no effect upon the O_2 uptake or the loss of N.

Messrs Oxo, Ltd. generously presented us with the dried post pituitary gland and Messrs Organon, Ltd. the desoxycorticosterone phosphate.

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THE EFFECT OF INTRODUCTION OF ISOTONIC SODIUM CHLORIDE SOLUTION INTO THE CISTERNA MAGNA OF THE DOG ON THE CELL CONTENT OF THE CEREBROSPINAL FLUID

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In a series of experiments on unanaesthetized dogs, in which the pressure of the cerebrospinal fluid was measured in the cisterna magna, Webster & Freeman (1941) found leucocytes in the cerebrospinal fluid in nearly all experiments in which isotonic sodium chloride solution was used to fill the recording manometer; these cells were not observed when the cerebrospinal fluid was allowed to rise into the empty manometer or when the manometer had previously been filled with cerebrospinal fluid. It is evident that the findings of these workers cast doubt on the validity of the results in many earlier experiments where isotonic sodium chloride solution was allowed to enter the subarachnoid space.

The following experiments were undertaken to determine whether the introduction of isotonic sodium chloride solution into the subarachnoid space of dogs anaesthetized with 'Nembutal' (sodium ethyl-methyl-butyl barbiturate) brings about any change in the cell content of the cerebrospinal fluid and, if a change is observed, to find a solution which does not produce such change.

Experimental procedure

Owing to war conditions little selection of dogs was possible. Diseased and grossly undernourished animals were always rejected. 'Nembutal' (Abbott), 40 mg./kg. body weight, was dissolved in 10 c.c. water and injected intraperitoneally. Whenever the dogs showed signs of recovery from the anaesthetic, an additional dose of nembutal was given in 2 c.c. water. A needle was introduced into the cisterna magna by cutting through the skin of the back of the neck from the inion to the level of the third or fourth cervical spine. The skin was then retracted and the muscles exposed. The surface of exposed muscle between the base of the skull and the second cervical spine was sterilized by cauterizing with a hot iron. A sterile short-bevelled needle (gauge 18 B.W.G.) with stilette was then introduced into the cisterna magna. After the needle had been successfully introduced the stillette was withdrawn and a small specimen of cerebrospinal fluid removed for cytological examination. A vertical glass manometer, 1·00 mm. in bore and 40 cm. in length, was connected with the needle by means of a metal adapter and a short piece of rubber tubing.

In one group of experiments the cerebrospinal fluid was allowed to rise into the empty manometer. In the rest of the experiments the manometer was filled initially with isotonic sodium chloride solution or with the solution under investigation. When it was desired to introduce a bigger volume of solution, the capacity of the manometer was increased by attaching a piece of fairly wide-bore rubber tubing to its upper end. This additional fluid entered the subarachnoid space during the course of 2 or 3 min. leaving in the manometer a short column of solution, rarely exceeding 0·05 c.c. in volume, to register the pressure of the cerebrospinal fluid. Additional fluid entered the subarachnoid space by diffusion probably aided by the normal rhythmic variations in the pressure of the cerebrospinal fluid. Whenever possible, experiments were continued for at least 5 hr. after the introduction of the needle into the subarachnoid space. A sample of cerebrospinal fluid was removed for examination at the end of the experiments. All specimens of cerebrospinal fluid, immediately after collecting, were examined in a Fuchs-Rosenthal counting chamber. Films were made of any specimen found to contain cells, and were stained with Leishman's stain. An experiment was discontinued if the initial specimen of cerebrospinal fluid contained more than twenty-five red blood corpuscles per cu.mm. Aseptic technique was used throughout the experiments, and apparatus was sterilized in an autoclave. Arterial blood pressure was recorded with a mercury manometer from a cannula in the femoral artery.

Results

The effect of 'Nembutal' anaesthesia on the cell content of the cerebrospinal fluid. The effect of nembutal anaesthesia on the cell content of the cerebrospinal fluid was studied in eight dogs. In this series of experiments the cerebrospinal fluid was allowed to rise into the empty manometer. The results of the experiments are summarized in Table 1. Neither nembutal anaesthesia nor the

TABLE 1. The effect of 'Nembutal' anaesthesia on the cell content of the cerebrospinal fluid of dogs

Exp. no.	Weight in kg.	Total wt. of 'Nembutal' administered in g.	Total duration in hr.	Cell content of cerebrospinal fluid: Initial P	Initial M	Initial E	Terminal P	Terminal M	Terminal E
1	7·5	0·46	3·8	0	0	0	0	0	0
2	11	0·66	3·3	0	190	0	0	0	0
3	10	0·72	5·3	0	0	0	0	0	0
4	10	0·56	5·0	0	0	0	0	0	0
5	10	0·64	5·0	0	30	0	0	0	0
6	11	0·70	5·8	0	0	0	0	0	0
7	9	0·54	6·3	0	20	0	0	0	0
8	7·5	0·39	6·0	0	40	0	0	0	0

P = polymorphonuclear leucocyte; M = mesothelial cell; E = erythrocyte.

presence of the needle in the cisterna magna had any influence on the cell content of the cerebrospinal fluid. In four of the experiments mesothelial cells were present in the initial sample of fluid removed immediately after puncture, but they were absent from all the terminal samples. The maximum number of mesothelial cells in any single experiment was 190/cu.mm. of cerebrospinal fluid.

The effect of the introduction of isotonic sodium chloride solution into the cisterna magna on the cell content of the cerebrospinal fluid. The effect of the introduction

of isotonic sodium chloride solution into the cisterna magna was studied in thirteen dogs. The results of these experiments are summarized in Table 2. The isotonic sodium chloride solution was prepared by dissolving sodium chloride A.R. in freshly distilled water to make a 0·9% (w/v) solution. The solution was sterilized in an autoclave. Polymorphonuclear leucocytes were present in the cerebrospinal fluid at the end of nine out of thirteen experiments.

TABLE 2. The effect of the introduction of isotonic sodium chloride solution into the cisterna magna on the cell content of the cerebrospinal fluid

Exp. no.	Weight in kg.	Total wt. of 'Nembutal' administered in g.	Vol. of isotonic NaCl solution introduced in c.c.	Duration in hr.	Cell content of cerebrospinal fluid: Initial P	Initial M	Initial E	Terminal P	Terminal M	Terminal E
1	8·0	0·57	1·6	5·75	0	0	0	1020	0	0
2	6·0	0·38	2·0	4·5	0	0	0	30	0	0
3	8·5	0·54	1·5	4·75	0	40	0	0	0	0
4	11·4	0·74	1·5	5·5	0	20	0	270	0	0
5	4·0	0·26	1·6	4·7	0	0	0	200	0	180
6	14·5	0·92	0·4	5·0	0	0	0	0	0	0
7	10·5	0·58	1·5	5·0	0	0	0	200	0	0
8	14·0	1·12	1·2	5·5	0	70	0	1370	0	0
9	8·0	0·64	2·0	4·5	0	0	0	270	360	0
10	6·0	0·43	2·0	4·5	0	0	0	0	0	0
11	9·5	0·58	0·4	4·5	0	0	0	0	0	0
12	13·0	0·83	2·0	5·5	0	40	0	600	0	0
13	8·7	0·75	2·4	5·5	0	0	0	200	0	0

P = polymorphonuclear leucocyte; M = mesothelial cell; E = erythrocyte.

The polymorphonuclear leucocytes were generally unaccompanied by other cells. In Exp. 9, however, a considerable number of detached mesothelial cells were also present, and erythrocytes accompanied the leucocytes at the end of Exp. 5 although absent at the beginning. Mesothelial cells were present in the initial sample of cerebrospinal fluid at the beginning of four experiments.

The effect of the introduction of Ringer's solution (Dale's formula) into the cisterna magna on the cell content of the cerebrospinal fluid. After the introduction of isotonic sodium chloride solution into the cisterna magna had been found to

TABLE 3. The effect of the introduction of Ringer-Dale's solution into the cisterna magna on the cell content of the cerebrospinal fluid

Exp. no.	Weight in kg.	Total wt. of 'Nembutal' administered in g.	Vol. of Ringer-Dale solution introduced in c.c.	Duration in hr.	Cell content of cerebrospinal fluid: Initial P	Initial M	Initial E	Terminal P	Terminal M	Terminal E
1	8·8	0·40	1·5	4·0	0	0	0	0	0	0
2	11·0	0·66	1·8	4·8	0	70	0	0	0	0
3	9·0	0·65	1·6	5·0	0	0	0	0	0	0
4	13·5	0·80	1·7	4·5	0	0	0	0	0	0
5	7·5	0·54	2·0	4·5	0	40	0	0	0	0
6	9·3	0·74	1·5	5·8	0	160	0	0	0	0

P = polymorphonuclear leucocyte; M = mesothelial cell; E = erythrocyte.

cause the appearance of leucocytes in the cerebrospinal fluid, an attempt was made to discover a solution which did not produce this effect. The above experiments were accordingly repeated using Ringer's solution—Dale's formula (Burn & Dale, 1922). It will be observed from Table 3 that, in a series of six experiments, the introduction of Ringer-Dale's solution into the subarachnoid space did not cause leucocytes to appear in the cerebrospinal fluid. Mesothelial cells were present in the specimen of cerebrospinal fluid removed at the beginning of three of the experiments but absent from the terminal sample.

The effect of the introduction of distilled water into the cisterna magna on the cell content of the cerebrospinal fluid. The effect of the introduction of distilled water into the cisterna magna was studied in eight dogs, and the results of the experiments are summarized in Table 4. Distilled water did not cause leucocytes to appear in the cerebrospinal fluid. In three experiments mesothelial cells were present in the initial sample of cerebrospinal fluid but absent from the terminal sample. In two experiments a few erythrocytes were observed at the beginning but were absent at the end.

TABLE 4. The effect of the introduction of distilled water into the cisterna magna on the cell content of the cerebrospinal fluid

					Cell content of cerebrospinal fluid					
					Initial			Terminal		
Exp. no.	Weight in kg.	Total wt. of 'Nembutal' administered in g.	Vol. of distilled water introduced in c.c.	Duration in hr.	P	M	E	P	M	E
1	5·6	0·25	1·8	4·0	0	0	0	0	0	0
2	6·0	0·48	2·5	5·0	0	110	0	0	0	0
3	8·0	0·51	2·0	4·5	0	45	0	0	0	0
4	12·0	0·77	2·5	5·7	0	250	20	0	0	0
5	8·2	0·53	1·8	4·5	0	0	0	0	0	0
6	6·0	0·38	2·5	5·3	0	0	0	0	0	0
7	5·7	0·36	2·0	4·8	0	0	0	0	0	0
8	9·6	0·61	2·2	4·8	0	0	15	0	0	0

P = polymorphonuclear leucocyte; M = mesothelial cell; E = erythrocyte.

DISCUSSION

It is evident from the above experiments that the introduction of isotonic sodium chloride solution into the cisterna magna is generally followed after 4–5 hr. by the appearance of polymorphonuclear leucocytes in the cerebrospinal fluid and that the presence of the recording needle in the cisterna magna for a similar period of time does not itself cause leucocytes to appear. These results confirm the findings of Webster & Freeman (1941). The maximum volume of isotonic sodium chloride solution introduced during their experiments was 0·6 c.c.; this is considerably less than the volume of solution generally introduced in the present experiments. Webster & Freeman unfortunately fail to state the duration of their experiments, although it is probable,

from a consideration of the records of related experiments, that they were continued for approximately 5 hr. Grant (1929), working on the unanaesthetized human subject, came to the conclusion that long-continued presence of a needle in the cisterna magna produces a sterile meningitis with an increased cell count in the cerebrospinal fluid, and that this is especially the case if the isotonic sodium chloride solution used to fill the manometer is allowed to mix freely with the cerebrospinal fluid. When isotonic sodium chloride solution was prevented from entering the subarachnoid space, the pleocytosis was not so frequently observed. These findings are not in entire agreement with those of Webster & Freeman (1941) nor with the results of the experiments now under consideration. Grant, however, neither states the duration of his experiments nor the number performed, nor makes any comment on the character of the cells present in the cerebrospinal fluid.

In the present experiments, introduction of isotonic sodium chloride solution into the cisterna magna was not invariably followed by the appearance of leucocytes in the cerebrospinal fluid; in four out of thirteen experiments no cells were found in the final sample of cerebrospinal fluid. In two of these experiments, however, a relatively small volume of isotonic sodium chloride solution had been introduced, and three of the experiments were of rather short duration. It will be noticed that mesothelial cells were present in a proportion of all the experiments immediately after cisternal puncture and, with one exception, were absent from the terminal samples of cerebrospinal fluid. These cells were presumably detached during the introduction of the needle; their identity was established by comparing them with cells obtained in scrapings from the pia-arachnoid. Their absence at the end of the experiments suggests that they had either been carried away from the cisterna by the normal circulation of the cerebrospinal fluid or had gravitated to some other part of the subarachnoid space.

When polymorphonuclear leucocytes appeared in the cerebrospinal fluid after the introduction of isotonic sodium chloride solution, they were generally unaccompanied by any other type of reactive cell. Webster & Freeman do not specify the character of the cells observed in their experiments but merely describe them as 'white blood cells'; this might include both polymorphonuclear leucocytes and lymphocytes. An interesting paper has recently appeared by Hammes (1944) on the reaction of the meninges to blood. This reaction may account for the cells observed in the present experiments. Hammes studied 114 fatal cases of intracranial aneurysm. He found that blood had a marked irritant action on the meninges. The earliest evidence of meningeal reaction was seen in cases which had survived for 2 hr. The evidence consisted of small collections of polymorphonuclear leucocytes around the pial vessels. After 4 hr. a more intense polymorphonuclear reaction set in and lymphocytes began to appear; these cells, like the polymorphs, were first seen around the

pial vessels. Even after 16 hr. the reaction was still predominantly polymorphonuclear. Only after 24 hr. were mesothelial cells found to take part in the reaction. Although blood may be a more powerful meningeal irritant than isotonic sodium chloride solution, it is probable that the same sequence in the outpouring of reactive cells takes place both with blood and with isotonic NaCl solution. The occurrence, therefore, of polymorphonuclear leucocytes unaccompanied by other reactive cells, in the present experiments, suggests that the experiments were terminated during the initial stage of meningeal reaction.

The absence of reactive cells in the cerebrospinal fluid after the introduction of distilled water was unexpected. It is interesting, however, to note that Weed (1935–6) has shown that distilled water and Ringer-Locke's solution of twice the usual concentration are absorbed from the subarachnoid space at the same rate as normal Ringer-Locke's solution; he suggests that the solutions are rapidly rendered isotonic within the subarachnoid space.

No claim, however, is made that either Ringer-Dale's solution or distilled water would have been found non-irritant if the experiments had been continued for a longer period of time. It is possible, too, that prolonged presence of the needle in the cisterna magna would itself ultimately have caused reactive cells to appear in the cerebrospinal fluid. The experiments do, however, seem to establish that isotonic sodium chloride solution is a much more powerful irritant to the meninges than either Ringer-Dale's solution or distilled water.

Summary

1. A study has been made of the effect of the introduction of isotonic sodium chloride solution into the cisterna magna on the cell content of the cerebrospinal fluid of dogs anaesthetized with 'Nembutal'. The duration of each experiment was approximately 5 hr.

2. Polymorphonuclear leucocytes, generally unaccompanied by other reactive cells, were present in the cerebrospinal fluid at the end of nine out of thirteen experiments.

3. The introduction, under the same conditions, of Ringer's solution—Dale's formula—or of distilled water into the cisterna magna did not cause reactive cells to appear in the cerebrospinal fluid.

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THE ACCURACY OF HAEMOGLOBIN DETERMINATION BY THE COPPER SULPHATE—BLOOD GRAVITY METHOD IN INDIAN SOLDIERS

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Phillips, Van Slyke, Dole, Emerson, Hamilton & Archibald (1945) described a simple technique for determining the specific gravity of whole blood and plasma. 'The method is based on the fact that plasma or whole blood dropped into a solution of copper sulphate of known gravity is encased in a sack of copper proteinate and the gravity of this discrete drop is not changed for about 15 sec. The rise or fall of the drop during this interval shows whether it is lighter or heavier than the solution.' Thus, by dropping blood or plasma into standard copper sulphate solutions with gravities graded at 0·001 intervals, the gravity can be determined to $\pm 0{\cdot}0002$. The solution does not significantly change its gravity until one-fortieth of its volume of blood has been added, so that 100 ml. of solution suffices for nearly 100 observations.

The originators showed that the gravity of plasma is directly related to the plasma protein content, and this remains the most useful application of the method. They also showed that the haemoglobin can be calculated from the equation

$$\text{Haemoglobin g./100 ml.} = H \times \frac{G_b - G_p}{G_c - G_p},$$

where H is the g. haemoglobin in 100 ml. of centrifuged cells, and G_b, G_p, and G_c are the specific gravities of the whole blood, plasma, and centrifuged cells respectively. By assuming normal values of 33·9 for H and 1·0970 for G_c the haemoglobin can be calculated from the blood and plasma gravities; and by further assuming a normal value of 1·0264 for the plasma gravity the haemoglobin can be calculated from the blood gravity alone. The originators claimed an accuracy of $\pm 2\%$ for the former method, and $\pm 10\%$ for the latter.

The purpose of this paper is to report on the reliability of the calculation of the haemoglobin from the blood gravity alone, for we have often felt the need of a rapid and reasonably accurate method of segregating the anaemic members of a population such as a large group of recruits.

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Material and methods

We measured the whole blood gravity, plasma gravity, haemoglobin content, and mean corpuscular volume of venous blood from 270 Indian soldiers, most of whom were healthy recruits. Anaemic bloods were obtained from hospital patients; most of these had the 'malnutrition-anaemia syndrome', but hypoproteinaemia (which would introduce an error into the calculation) was rarely seen.

The work was carried out during the cold weather of 1944–5, and the laboratory temperature varied between 18 and 24° C.

Blood. Venous blood was withdrawn without stasis in syringes sterilized by hot liquid paraffin, and 2·5 ml. were carefully measured into a tube containing 5 mg. of Wintrobe's oxalate mixture. Most determinations were done about 1 hr. after the blood had been collected. The blood in the tubes was well mixed before use.

The very anaemic bloods sedimented very rapidly even in the capillary pipettes, so that successive drops had increasingly greater gravities. It was necessary to mix the blood well before withdrawing each test drop.

A trace of haemolysis did not seem to interfere with the readings.

There was no significant change in either blood or plasma gravity estimated at 2 and 7 hr. after withdrawing the blood.

Copper sulphate solutions. Stock solutions of copper sulphate of specific gravity 1·100 were made by dissolving 159·63 g. of $CuSO_4.5H_2O$ in distilled water to a final volume of 1000 ml.* The specific gravity of these solutions after filtration was determined by comparing the weight of 100 ml. with that of 100 ml. water. It was found to be 1·100 ± 0·0003.

Standard copper sulphate solutions with gravities graded at 0·001 intervals from 1·015 to 1·074 were made by diluting the appropriate volume of stock solution in a 100 ml. flask. The number of ml. of stock solution needed to make 100 ml. of any standard solution is 1 less than the number indicated in the second and third decimal places of the desired gravity. For example, to prepare a solution with gravity 1·049 dilute 49 − 1 = 48 ml. of stock solution to 100 ml.

The standard solutions were stored in 6 oz. screw-topped flat bottles.

We used distilled water, but were able to confirm the originators' claim that tap water gives accurate solutions.

Haemoglobinometer. We used the Zeiss 'Hemometer' which has a glass wedge standard. This instrument was restandardized against bloods of known haemoglobin content measured as alkaline haematin against a standard solution made from pure crystalline haemin (B.D.H.) (King, Gilchrist & Delory, 1944).

Gravity determinations. Small drops of blood or plasma from a capillary pipette were dropped into the appropriate solutions. The behaviour of the drop during the first 10 sec. was observed. If it remained stationary in a particular bottle it was of that gravity; if it rose and fell in adjacent bottles its gravity was easily estimated to the nearest 0·0002 by noting the relative speed of rise and fall.

Results

The relation between the haemoglobin content and gravity of oxalated blood is shown in Fig. 1. It will be seen that the relation follows the same trend in normocytic, macrocytic, and microcytic bloods. The amount of oxalate used increased the gravity by 0·0008, and this figure should be subtracted from the observed gravity to give the true blood gravity.

* The originators now recommend 170 g. copper sulphate to 1002·4 g. water, which gives more accurate results when the working temperature is below 20 or over 40° C.

Fig. 2 shows a curve drawn to fit these results (after correcting for oxalate) compared with the line given by the originators' equation which reduces to:

$$\text{Haemoglobin g./100 ml.} = 480{\cdot}18 \times \text{blood gravity} - 492{\cdot}86.$$

The discrepancy between the two curves varies up to 1 g. haemoglobin.

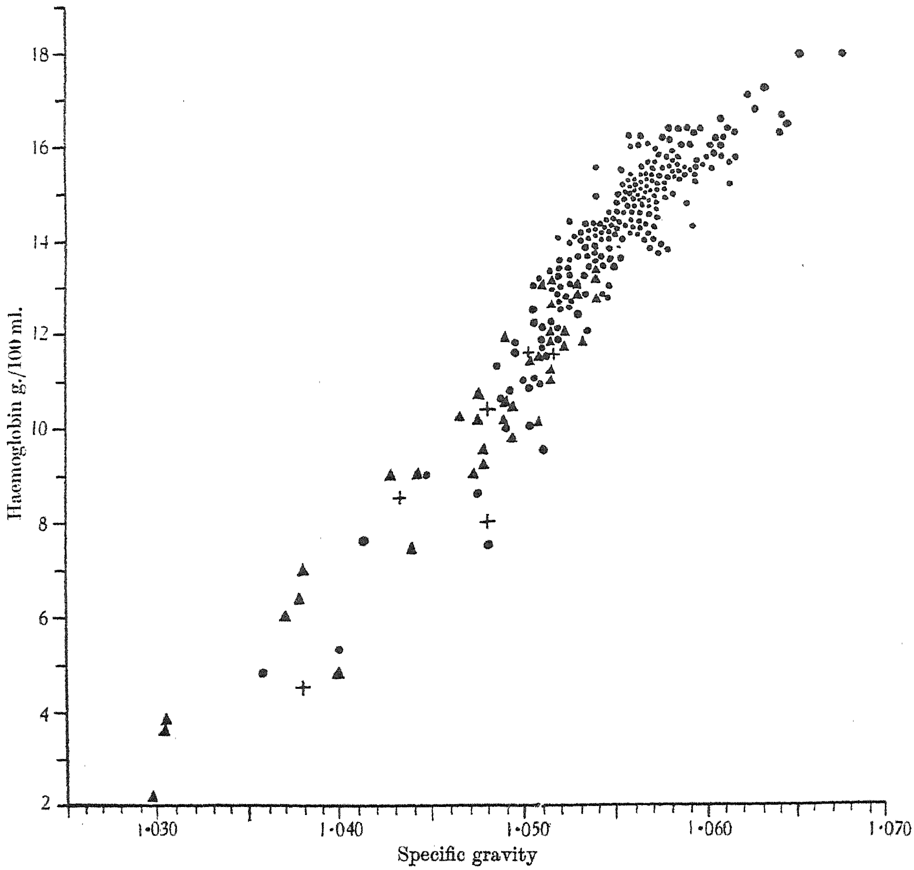

Fig. 1. The haemoglobin content and specific gravity of 270 specimens of oxalated blood from Indian soldiers. ▲ Macrocytic (m.c.v. over 100 cu.μ). • Normocytic (m.c.v. 80–100 cu.μ). + Microcytic (m.c.v. under 80 cu.μ).

The regression line to the authors' data ($\text{Hb} = 455{\cdot}85G_b - 466{\cdot}63$) is also shown; it suggests that the relation between haemoglobin and blood gravity is not linear.

Table 1 shows the mean and standard deviation (s) of the differences between the observed haemoglobin and the value calculated by the authors' curve from the gravity of whole blood (corrected for oxalate). We may deduce that in 95% of cases we may estimate the haemoglobin from the blood

gravity to within $\pm 1 \cdot 5$ g. when the haemoglobin is above 11 g., and to ± 2 g. when the haemoglobin is below 10 g. These are wide limits, but not so wide as to invalidate the method for rapid group examinations (see below).

Combined blood and plasma gravity estimations. On seventy-one bloods we measured the gravity of serum as well as of whole blood and plasma. Table 2

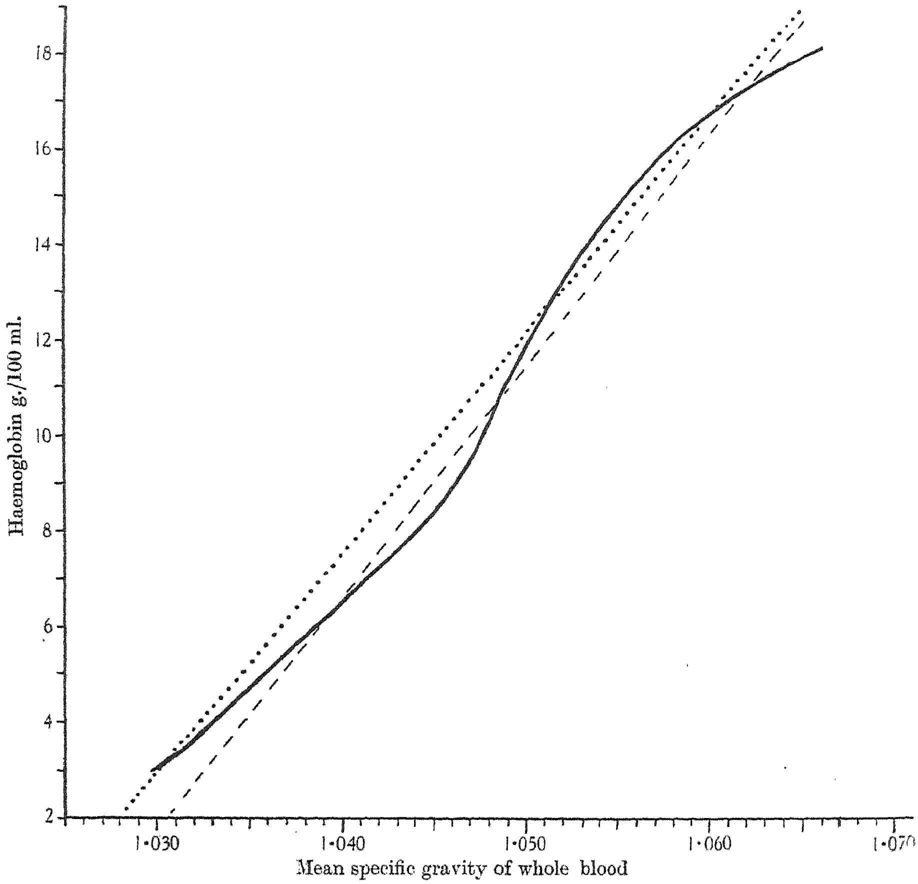

Fig. 2. The relation between the haemoglobin content and specific gravity of whole blood. —— Authors' curve. ---- Originators' curve. ···· Regression line to authors' data.

shows the mean and standard deviation of the differences between the observed haemoglobin and the value calculated according to the originators' equations from the whole-blood gravity, the blood and plasma gravities, and the blood and serum gravities. In the third calculation G_p was taken as serum gravity $+ 0 \cdot 0005$, the originators' correction.

It will be seen that introducing the serum or plasma gravity into the haemoglobin calculation substantially reduced the error, but it remained far too wide to justify the extra labour.

TABLE 1. The difference between the observed haemoglobin and the value calculated by the authors' curve from the whole blood gravity (corrected for oxalate)

Haemoglobin group	No. of cases	Difference between observed and calculated haemoglobin			
		Mean (g.)	s	Maxima (g.)	
Under 7 g.	9	+0·056	0·9419	−1·0	+1·4
7–8·9 g.	7	+0·001	1·1623	−1·5	+1·5
9–9·9 g.	8	−0·025	1·0173	−1·2	+2·0
10–10·9 g.	12	−0·025	0·8654	−1·1	+1·6
11–11·9 g.	21	−0·019	0·8134	−1·4	+1·5
12–12·9 g.	24	+0·105	0·6849	−1·1	+1·4
13–13·9 g.	35	−0·023	0·8966	−1·6	+1·8
14–14·9 g.	62	−0·019	0·7022	−1·9	+1·7
15–15·9 g.	63	+0·003	0·5550	−2·0	+1·4
16–16·9 g.	25	−0·016	0·7180	−1·4	+1·2
17 g. and over	4	−0·075	—	−0·3	+0·2

TABLE 2. The difference between the observed haemoglobin and the values calculated by the originators' equations from the blood, plasma, and serum gravities. Seventy-one cases

Haemoglobin calculated from	Difference between observed and calculated haemoglobin			
	Mean (g.)	s	Maxima (g.)	
Blood gravity	−0·558	0·9441	−2·2	+2·6
Blood and plasma gravity	−0·499	0·6650	−1·8	+1·7
Blood and serum gravity	−0·410	0·6980	−1·7	+1·7

We gained the impression that the measurement of serum gravity is technically easier and more accurate than that of plasma gravity. In ten of these bloods the plasma gravity, after making the oxalate correction, was actually less than the serum gravity—suggesting a negative fibrinogen value! The error probably lay in the plasma gravity.

The mean value of the oxalated plasma gravity (uncorrected) was 1·02706 ($s=0{\cdot}00198$). The originators' most recent equation is:

$$\text{Plasma protein} = 360 \times (\text{plasma gravity} - 1{\cdot}0070).$$

Accordingly (after deducting 0·0008 for the oxalate correction) the mean plasma protein in this series was 6·93 g. ($s=0{\cdot}713$ g.).

The mean value of the serum gravity was 1·02552 ($s=0{\cdot}00204$), corresponding to 6·67 g. serum protein ($s=0{\cdot}734$).

The mean value of the difference between the gravities of oxalated plasma and serum was 0·001578 ($s=0{\cdot}00077$), corresponding (after deducting 0·0008 for the oxalate correction) to a plasma fibrinogen of 0·280 g. ($s=0{\cdot}277$).

There was no statistical correlation between anaemia and plasma proteins—the correlation coefficient between haemoglobin and serum gravity was only 0·062, giving $t=0{\cdot}516$, $P=0{\cdot}6$. On the other hand, the five bloods with definite hypoproteinaemia (plasma protein under 5·6 g.) all had less than 11 g. haemoglobin per 100 ml.

Practical suggestions

Although this method is less accurate than the Sahli haemoglobin technique, it has the advantage of great rapidity. To pick out the anaemic members of a group it is only necessary to put one drop of blood from each person into one copper sulphate solution corresponding to the haemoglobin value chosen as the limit. If the drop rises, the individual is counted as anaemic. Table 3 shows the gravity of the copper sulphate solution corresponding to different haemoglobin values.

TABLE 3. The mean specific gravity of whole blood at different haemoglobin levels

Haemoglobin (g.)	4	6	8	10	11	12	13	14	15
Whole blood gravity	1·033	1·0387	1·0442	1·0478	1·0490	1·0505	1·0518	1·0536	1·0557

It may easily be calculated that if, for example, all bloods with a gravity under 1·049 be classified as having less than 11 g. haemoglobin, then about 30% of individuals with a true haemoglobin from 10 to 10·9 g., and about 4% with 9–9·9 g., will be placed in the 'over 11 g.' group. Similarly, 30% of people with 11–11·9 g. and 4% with 12–12·9 g. will be wrongly classified as under 11 g. Even the 30% of individuals with haemoglobin 11–11·9 g. wrongly classified will be a small fraction of most populations, and few errors of grouping will be made. In the present series, for example, out of 270 bloods only four with a blood gravity greater than 1·049 had under 11 g. haemoglobin, and only four with a blood gravity under 1·049 had over 11 g. haemoglobin.

SUMMARY

1. The specific gravity of the whole blood, measured by the copper sulphate technique, and the haemoglobin of 270 Indian soldiers were determined.

2. A curve has been drawn to show the mean whole-blood specific gravity at different haemoglobin levels. It is independent of the type of anaemia.

3. From this curve the haemoglobin may be calculated from the specific gravity of whole blood to within $\pm 1\cdot5$ g. if the haemoglobin is over 11 g., and to within ± 2 g. if it is below 10 g.

4. The utility of the method is that individuals from a large group can very quickly be classified as above or below a particular haemoglobin level by observing whether a drop of their blood sinks or rises in the copper sulphate solution of corresponding specific gravity.

5. By this method 70% of people with haemoglobin within 1 g. of the chosen level, and the great majority of those beyond these limits, will be correctly classified.

6. The measurement of serum gravity is more accurate than that of plasma gravity, but in spite of theoretical considerations the use of either, together with blood gravity, to calculate the haemoglobin, does not produce a sufficient increase in accuracy to justify the extra labour.

Our thanks are due to the Director of Medical Services in India for permission to publish this paper.

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URINE ACIDITY IN ALCOHOL DIURESIS IN MAN

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Available evidence suggests that the diuresis following ingestion of ethyl alcohol is of the same nature as water diuresis mediated by the pituitary gland (Eggleton, 1942*b*). In an effort to establish further points of similarity or difference between the two types of diuresis, a class experiment on the effects of exercise on urinary excretion was performed identical with those previously reported (Eggleton, 1942*a*, 1943) except for the substitution of alcohol as the diuretic in place of water or tea. The main results of the experiment confirmed those obtained when water had been used as the diuretic, but one unexpected difference suggested that alcohol *per se* might lead to the excretion of an acid urine. The matter has therefore been investigated in a number of subjects by observing the *p*H of the urine during water diuresis and during alcohol diuresis under strictly comparable conditions.

Methods

The class experiment was performed in the manner already described (Eggleton, 1942*a*), 40 g. alcohol in 250 c.c. solution being taken as the diuretic and exercise superimposed when the diuresis was well established. In the remaining experiments, each subject performed the two experiments (water ingestion 560 c.c., and alcohol ingestion 40 g. in 200 or 250 c.c., respectively) at the same time of day, usually on two successive days; in three cases, water was taken on the first day, and in four cases, alcohol. The same preliminary routine was followed on the two occasions: overnight fasting followed by an experiment in the morning, or an afternoon experiment following a 6 hr. fast, on each occasion a glass of water being taken $2\frac{1}{2}$ to 3 hr. beforehand.

Immediately the urine samples had been collected and measured, the *p*H was determined by the simple comparator, using the indicator range phenol red, brom-thymol-blue and methyl red. Readings could be made within 0·1 unit.

Results

I. *Class experiment on the effect of exercise during alcohol diuresis*

The average changes in the urine of eight subjects following a 60 sec. sprint during an alcohol diuresis are shown in Fig. 1, together with the results, reported earlier (Eggleton, 1942*a*), of a similar experiment made during water diuresis. On the main points of difference observed in such experiments with

water and with tea as the diuretic (Eggleton, 1943). the alcohol is seen to behave in the same way as water; both chloride and total N output remain far below the resting values by the end of the experiments. The results also suggest that vaso-constriction in the kidney (as indicated by a fall in the creatinine excretion), during or immediately following the exercise, was much less pronounced when alcohol was used as the diuretic. This is probably connected with the known fact that alcohol is a vaso-dilator, and with the observed fact that the exercise taken was not so violent; the subjects were clearly incapable, after taking alcohol, of making so great an effort as those

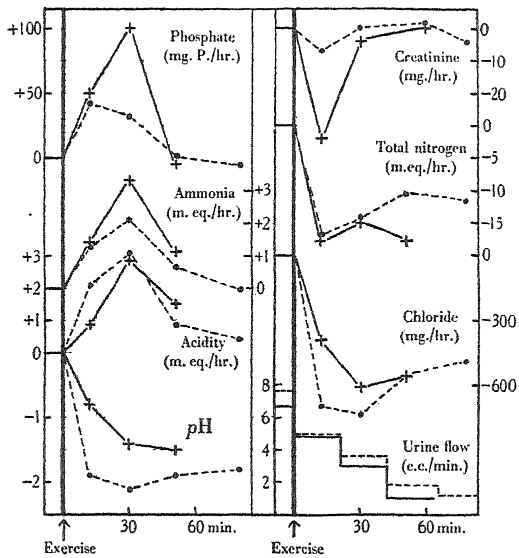

Fig. 1. Average changes in urinary excretion following exercise during alcohol and during water diuresis. •------• Alcohol diuresis (average of eight subjects). +——+ Water diuresis (average of seven subjects).

who had previously ingested only water. In spite of this, the results suggest that, with alcohol as the diuretic, the output of titratable acidity was rather greater, that of ammonia rather less, and the *p*H lower than when water was used as the diuretic. The differences are not statistically significant (the two experiments were performed on two different groups of individuals at an interval of two years, and all analyses were made by students), but appeared sufficiently suggestive to warrant a further, more careful, examination. It had been noted also that a change towards acidity occurred in the urine after alcohol ingestion before the exercise was taken, the effect being most definite in two subjects who, from symptoms and subjective sensations, were the most rapid absorbers.

II. *Comparison of alcohol diuresis with water diuresis in the same subjects*

Of the many factors known to affect urine *p*H, as many as possible were eliminated by comparing the effect of alcohol with that of water at the same time of day, under the same conditions of fasting and body hydration, and at similar rates of urine flow. The necessity for such precautions is demonstrated by the results shown in Fig. 2. In one subject, the urine *p*H, starting at 8·2, fell to 7·0 with the onset of diuresis and remained at that value throughout the experiment; in two subjects, the low initial *p*H (5·0 and 5·7 respectively) rose sharply with the onset of diuresis, and fell again later; and in the four

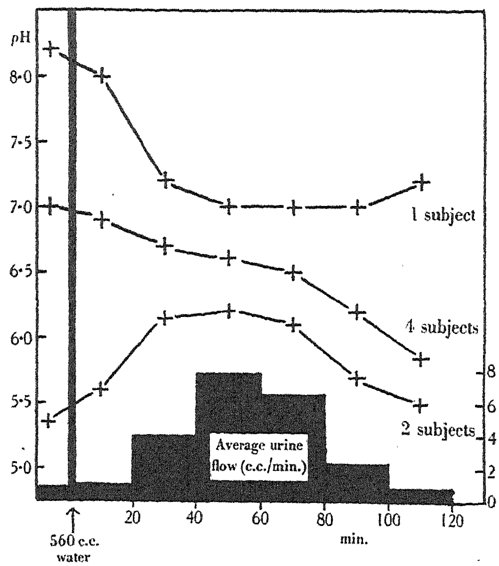

Fig. 2. Changes in urine *p*H during water diuresis in different subjects.

subjects starting at *p*H 6·9 or 7·0 there was a steady drift downwards, the *p*H falling more sharply as the diuresis died away. The success of the precautions taken is shown by the fact that, in only one of the subjects investigated, did the initial *p*H on the two days of experiment differ by more than 0·5 unit, the average difference in the remainder being 0·25 unit. The results of this anomalous subject were therefore discarded. The remaining seven sets of values come from six subjects, on one of whom a pair of morning and a pair of afternoon experiments were performed.

In every subject the *p*H of the urine after ingestion of alcohol was lower than that observed during the water diuresis, although the difference varied widely from one subject to another. The greatest difference occurred 40–60 min. after the drink, at which time the *p*H was, on the average, 0·9 unit lower in

the alcohol than in the water experiment. These results are summarized in Table 1.

TABLE 1. The change in urine pH during water diuresis and during alcohol diuresis in different individuals

	Water diuresis			Alcohol diuresis		
Subject	Initial pH	After 40–60 min.	Change	Initial pH	After 40–60 min.	Change
F. (M.)	8·2	7·2	−1·0	8·0	6·6	−1·4
B. (A.)	6·9	6·8	−0·1	6·4	5·3	−1·1
E. (A.)	7·0	6·4	−0·6	7·4	6·2	−1·2
G. (M.)	7·0	7·1	+0·1	6·7	4·8	−1·9
J. (A.)	7·0	6·7	−0·3	7·0	6·2	−0·8
A. (A.)	5·7	6·6	+0·9	5·7	5·8	+0·1
G. (A.)	5·0	5·7	+0·7	5·4	5·1	−0·3
		Average	−0·05			−0·95

(M.) = morning experiment; (A.) = afternoon experiment.

In five of the seven alcohol experiments, the early fall in urine pH was not maintained, although the urine remained more acid than that secreted during the water diuresis. This effect is seen when the average curve for the seven

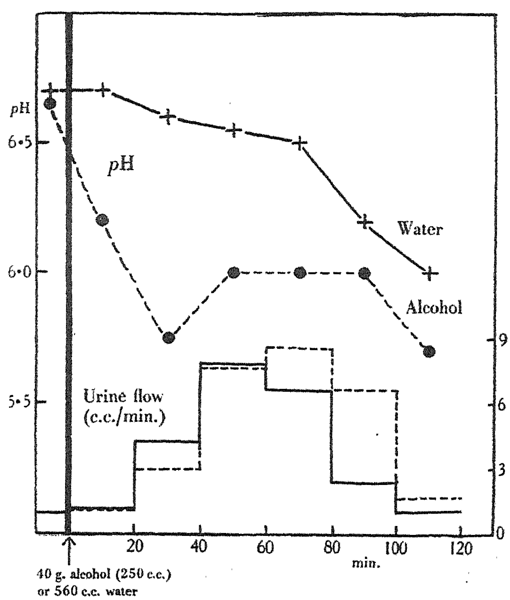

Fig. 3. The average changes in urine pH during water diuresis and during alcohol diuresis in six subjects.

alcohol experiments is compared with the average curve of the seven experiments with water (Fig. 3). The average rates of urine flow in the two sets of experiments are also shown. They are not dissimilar, but the alcohol diuresis is slightly later in onset than the water diuresis and is somewhat greater in

magnitude. The average output of urine in 2 hr. following ingestion of 560 c.c. water was 485 c.c. (varying from 310 to 630 c.c.), and that following ingestion of 40 g. alcohol in 200 or 250 c.c. was 630 c.c. (varying from 520 to 790 c.c.).

III. *Possible causes of increased urine acidity during alcohol diuresis*

(*a*) *Excretion of acetic acid.* In attempting to find the cause of this increased acidity of the urine during an alcohol diuresis, attention was first directed to the possible excretion of acetic acid. This substance is known to be produced by the liver during the metabolism of alcohol (Lundsgaard, 1938; Leloir & Muñoz, 1938), but it has not been detected in the blood stream (Himwich, Nahum, Rakieten, Fazikas, Du Bois & Gildea, 1933) although some workers (quoted by Sendroy, 1938) have observed a tendency to acidosis of metabolic type, with alkali deficit, both in chronic and in experimental alcoholism. The specific lanthanum test (Krüger & Tschirch, 1929) was used for its detection.

According to the authors of this test it should yield a positive colour with amounts of acetate as small as 0·1 mg. (in 1–3 c.c. of solution), but under present conditions, a definite positive result could not be obtained with less than 0·5 mg. Moreover, this degree of sensitivity was considerably reduced when the acid had first to be distilled from urine. Other volatile substances (not alcohol itself) interfered with the reaction, whether HCl or H_2SO_4 were used to acidify the urine, and no definite positive test could be obtained on the concentrated distillate if less than 10 mg. acetic acid had been present in the 50 c.c. urine distilled.

The test was applied in three separate experiments on a subject known to absorb alcohol rapidly. The urine sample collected during 30–45 min. following the ingestion of 1 g. alcohol/kg. registered the lowest *p*H in each experiment and was therefore used for the test. This was unequivocally negative in all three experiments. If any acetic acid was excreted, less than 10 mg. were present in the urine secreted during the early stages of the alcohol metabolism, i.e. less than 1/5000th part of the alcohol ingested.

On several occasions also the acid urine secreted immediately after ingestion of alcohol was tested for the presence of aceto-acetic acid by Rothera's reagent. The only positive result recorded was a very faint one, considerably less than that given by 1 : 100,000 dilution of aceto-acetic acid.

(*b*) *Reduced excretion of ammonia.* The second possible cause of the increased acidity of the urine after ingestion of alcohol, suggested by the results of the class experiment, was a diminished production of ammonia by the kidney, but any direct attack on the problem was rendered difficult owing to lack of knowledge concerning the factors involved in the production of ammonia. From long-term experiments on the acidosis produced by fasting, the conclusion has been drawn that 'ammonia production follows the induction of acidosis only after an interval of hours or days' (Smith, 1937); yet it is clear from the results presented in Fig. 1 that, with the acidosis resulting from exercise, no appreciable lag in ammonia production is discernible. Hubbard (1923) came to the conclusion that the *concentration* of ammonia in the urine was related to its *p*H, and that, at constant *p*H, the rate of ammonia

excretion was related to rate of fluid output. His results, obtained on one human subject by varying food and fluid intake, did not agree with those obtained by Marshall & Crane (1922) when the rate of flow was varied in the dog by section of the sympathetic supply to one kidney; they found no correlation between water and ammonia output at constant *p*H. It seemed advisable, therefore, to observe the ammonia concentration and total output over a wide range of flow and of *p*H before coming to any conclusion in regard to the possible action of alcohol, and with this end in view the study was restricted in the main to observations on one subject.

The quickest method of ammonia estimation is titration of the neutralized urine (phenolphthalein faint pink) after addition of neutralized formol, but this titration value also includes the amino-acids present. In several experiments a comparison was made of the results obtained by this method and by the aeration method in which ammonia only is estimated; the results of one such experiment are shown in Fig. 4. The amino-acid fraction varied from 0·16 m.eq./hr. at slow rates of urine flow to 0·28 m.eq./hr. at fast rates. On another occasion a variation from 0·26 to 0·51 m.eq./hr. was observed in the same subject, and one from 0·12 to 0·44 m.eq./hr. in a second subject. This range of variation, partly caused by the analytical errors inherent in the methods, is small in relation to the observed changes in ammonia excretion, and the main body of results has been obtained by use of the formol titration method. The relative inaccuracy of the end-point in the titration is offset by the fact that a reasonably high titration value can be obtained under all conditions by varying the volume of urine used, from 5 c.c. of a concentrated urine, diluted to 50 c.c. before titration, to 50 c.c. of the most dilute urine.

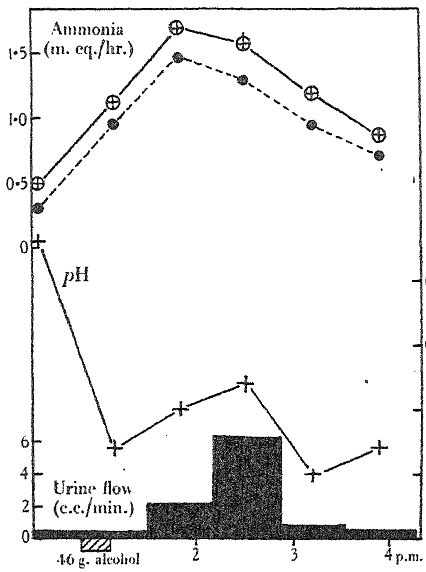

Fig. 4. The relationship between ammonia excretion and urine *p*H after ingestion of alcohol. ⊕——⊕ Ammonia + amino-acids (formol titration). ●------● Ammonia (aeration method).

It will be noted in Fig. 4 that it is the ammonia output and not its concentration which has been plotted, and that this output varies roughly inversely with the urine *p*H. The correlation is not absolute: e.g. the sudden increase of rate of flow from 0·4 to 2·0 c.c./min. (a 5-fold change) is accompanied by a 50% increase in ammonia output although the *p*H has risen

0·3 unit, and the equally sudden decrease in flow from 6·0 to 0·8 c.c./min. is accompanied by a 25% decrease in ammonia output although the pH has fallen by 0·7 unit. In this particular experiment, the ammonia concentration also varies inversely with the pH, but the relationship is fortuitous. The results of another alcohol experiment plotted in Fig. 5 show again the fairly close inverse relationship between ammonia output and pH, and in addition the entire lack of correlation between ammonia *concentration* and pH. On the

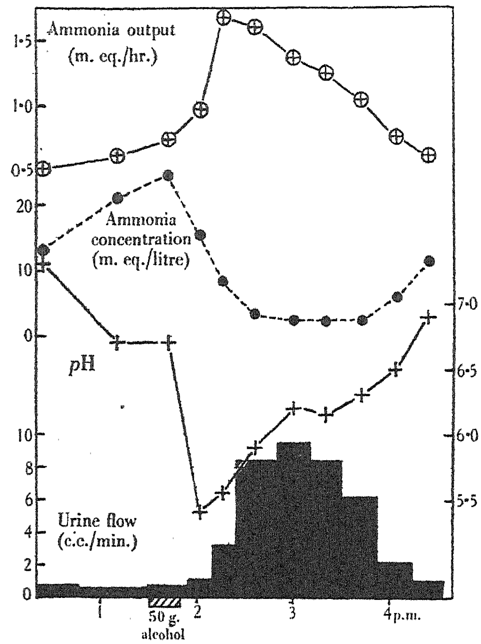

Fig. 5. Showing the lack of consistent relationship between ammonia *concentration* and urine pH during alcohol diuresis.

basis of this and many similar results, a graph has been constructed to show the relation between ammonia output and urine pH under resting conditions, during water diuresis, and during alcohol diuresis (Fig. 6). The scatter of the points is due largely to the variations in rate of urine flow (a 40-fold range), those to the right of the line occurring during diuresis, those to the left at low rates of flow. There is no indication that the ammonia output after ingestion of alcohol is less than that occurring under other conditions.

Discussion

The observed reduction in urine pH following the ingestion of alcohol is an unexpected result which is not easily explained. The well-known action of alcohol in stimulating gastric secretion would, in fact, have led one to expect

a change in the reverse direction. The increased acidity observed is not due to excretion of acetic acid as was at first surmised, nor is it due to a diminished production of ammonia, as the results of the class experiment on the effects of exercise had tentatively suggested. The only remaining possibilities would seem to be either the excretion of some acid product of metabolism other than

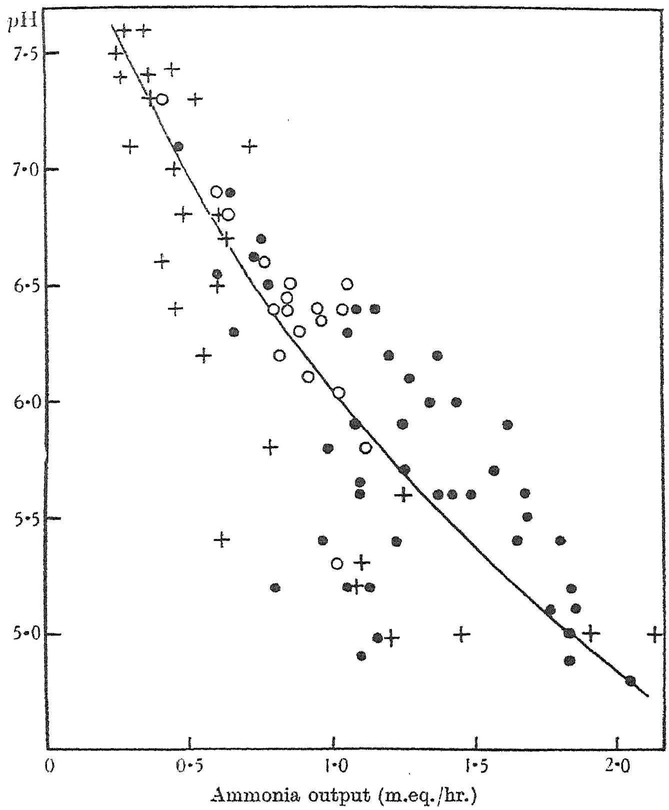

Fig. 6. The relationship between ammonia excretion and urine pH under resting conditions (+), during water diuresis (o) and during alcohol diuresis (•) in one subject: covering a 40-fold range in rate of urine flow.

acetic acid, or an acidity induced by some chain of events connected with the physical properties of alcohol, including its rapid rate of absorption. Any conclusion as to its mode of action, therefore, must await more detailed knowledge of possible different types of urine acidity induced by such agents.

Summary

1. A short period of severe exercise superimposed on an alcohol diuresis produces essentially the same effect on the urine as that observed during a water diuresis (Fig. 1).

2. During a water diuresis (uncomplicated by exercise), the *p*H of the urine rises from a low resting value and falls from a high resting value (Fig. 2).

3. In each of six subjects, the urine was consistently more acid during an alcohol diuresis than during a water diuresis (Fig. 3, Table 1).

4. This increased acidity of the urine in alcohol diuresis was not due to excretion of acetic acid, or of aceto-acetic acid.

5. An inverse relationship between urine *p*H and rate of ammonia excretion was observed, the result being unaffected by inclusion of amino-acids with the ammonia (Fig. 4).

6. The relationship was also relatively unaffected by large changes in rate of urine flow, and no consistent relationship was found between urine *p*H and ammonia concentration (Fig. 5).

7. The relationship between ammonia output and urine *p*H observed in resting samples and during water diuresis was not disturbed by ingestion of alcohol (Fig. 6).

I am indebted to Miss I. G. Smith for assistance with the ammonia determinations by the aeration method.

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THE EFFECT OF LOCAL TEMPERATURE ON FLUID LOSS IN THERMAL BURNS

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The rapid outpouring of plasma from the blood stream into the affected area is one of the most striking and important features during the first 48 hr. after thermal injury. The loss of fluid may be sufficient to cause shock and even death from circulatory collapse, if the area of the burn is sufficiently great, since the compensatory withdrawal of fluid from the undamaged tissues, although considerable, is not sufficient to prevent a fall in plasma volume during the early period of rapid oedema formation. This upset in the water balance of the body, resulting from the local damage to the capillaries, is greatest during the first 18 hr., and usually ceases at about 48 hr. The literature on this subject of fluid loss after thermal burns has in recent years been reviewed by Harkins (1938, 1942, 1944), and will not be repeated here.

The main treatment of thermal burns, as far as the plasma loss and shock are concerned, is replacement by plasma or serum transfusions. These transfusions will probably increase the local oedema but, if given repeatedly, generally restore the plasma volume. More recently attempts have been made in experimental animals and in man to lessen the degree of plasma loss by applying pressure to the injured part (Barnes & Trueta, 1941; Glenn, Gilbert & Drinker, 1943; Rossiter, 1944; Cameron, Allen, Coles & Rutland, 1945; Cope & Rhinelander, 1943). This pressure-bandaging increases the effective tissue tension and so tends to prevent the escape of plasma from the injured capillaries.

The experiments to be described below have also been designed to show whether the production of oedema can be lessened by keeping the affected areas cold as compared with keeping similarly affected areas warm. It was thought that the amount of oedema formation would depend upon the quantity of blood flowing through the damaged capillaries. Usually, in the treatment of burns, the affected part is kept warm with a resultant good blood flow. By keeping the local area cold, it was hoped to decrease the blood flow and so lessen the degree of fluid loss, especially in the early stages when the outpouring of plasma is so rapid.

Methods

General. Rabbits, dogs and goats have been used in this investigation. In all animals the legs have been the part burned. Before scalding, the legs in all animals were clipped.

Rabbits were anaesthetized with nembutal intravenously, and the hindlegs (one or both as stated) scalded in a beaker of water at 75° C. for 45 sec. The legs were always immersed to approximately the same depth, viz. just below the knee joint. This has been the standard burn for rabbits used throughout this investigation. Except in those cases where stated, the rabbits, after burning, were allowed to recover from the anaesthetic, which usually took 1–2 hr. When they had recovered they were given greens to eat and water to drink.

In the various experiments the hindlegs were kept at a constant temperature after burning, 0, 37, or 45° C., for periods of 2, 6, 12, 24 or 48 hr. This was done as follows: A 6 in. wide plaster of Paris bandage was placed around the posterior part of the rabbit's abdomen. This prevented the rabbit from flexing its hip joint, and as a rabbit does not flex its knee joint with the hip joint in extension the two hindlegs were kept fairly straight and not flexed under the abdomen. The rabbit was then put in a rabbit box with a hole in the posterior end through which protruded the hind part of the animal. After recovery from the anaesthetic, the rabbit remained in the box quite happily with its hindlegs hanging in a water-bath kept at the required constant temperature. Each leg was placed in a cylindrical rubber bag after burning, so that the legs were kept reasonably dry.

Goats were anaesthetized with nembutal intravenously, and the legs burned by standing them in drums containing water at 85° C. for 2½ min. They recovered from the anaesthetic in about ½ hr. The legs were kept at the required temperature by placing them in drums of water at that temperature. The legs on one side of the goat were kept in ice-water and those on the other side at room temperature or in water at 37° C.

Dogs were anaesthetized with nembutal intravenously and kept under the anaesthetic throughout the experiment. The paws (fore or hind) of the dog were scalded in water at 90° C. for 2 min. or 80° C. for 1½ min. Dogs have been used for the measurement of the lymph flow and blood flow in the burned paw when kept at various temperatures afterwards.

In the investigation of the lymph drainage of the burned area, the main lymph duct of the foreleg was cannulated just above the paw, and in the hindleg one of the lymph ducts running alongside the external saphenous vein was cannulated, the other being ligatured. Dry heparin was placed in the cannula at intervals to prevent coagulation. Samples of lymph were collected at various times before and after burning, as described below.

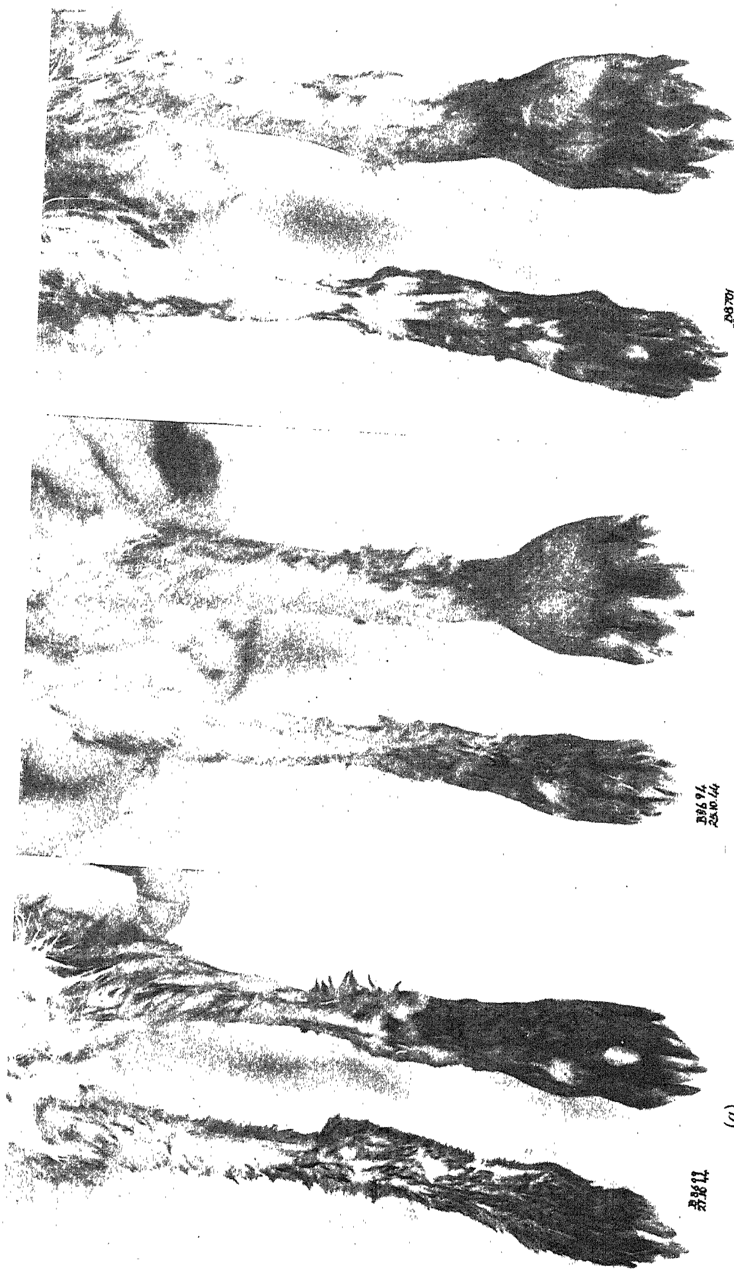
The blood flow of the forepaw in the dog was estimated simply by inserting a Y-shaped cannula in the course of the large vein draining the foreleg just above the paw. The blood could be allowed to circulate normally or, by clipping the proximal end of the vein and unclipping the side tube, the blood flowed along the latter into a 25 c.c. pipette. The time taken to fill the pipette was noted and the rate of blood flow estimated. The blood was then reintroduced into the circulation. In these experiments the dog was supported in the prone position on a frame with the forelegs hanging down. The pipettes were sloped at an angle roughly parallel to the forelegs and were fixed in position. The pressure of the column of blood in the pipette was therefore kept at a minimum and the same throughout the experiment. The blood was made incoagulable by the intravenous injection of heparin, 300 units per kg., followed 1½ hr. later and then hourly by 150 units per kg.

Analytical methods. (*a*) Haemoglobin was estimated by the Haldane haemoglobinometer.

(*b*) Plasma and lymph protein concentrations were determined by micro-Kjeldahl digestion and Nesslerization.

(*c*) Plasma non-protein nitrogen (N.P.N.) was determined by precipitation of the proteins with trichloroacetic acid, micro-Kjeldahl digestion and Nesslerization.

(*d*) Body temperature was measured by clinical thermometer. The temperature of the rabbits was recorded in the mouth between the cheek and the gums, since the rectal temperature might



The general appearance of the effects of local temperature on the amount of oedema formation in the scalded hindlegs of rabbits. For explanation see text (p. 323).

To face p. 323

be affected by the temperature of the water-bath into which the hindlegs were immersed. In ten normal rabbits it was shown that the mouth temperature was consistently a little lower than the rectal temperature, 0·5° F. on the average. Since rabbits do not breathe through the mouth, the mouth temperature should give a good indication of the general body temperature.

(*e*) Blood pressure was measured in rabbits by the Grant-Rothschild method (Grant & Rothschild, 1934). Great care was taken to have the rabbit quiet and the ear artery completely dilated by warming. Each determination of the blood pressure is the mean of ten readings.

(*f*) The oxygen content of the blood was determined with the van Slyke manometric apparatus. For these estimations the blood was withdrawn under liquid paraffin, and the analyses begun within the next 3 hr.

In the rabbits, all blood samples were taken from the ear vein.

Results

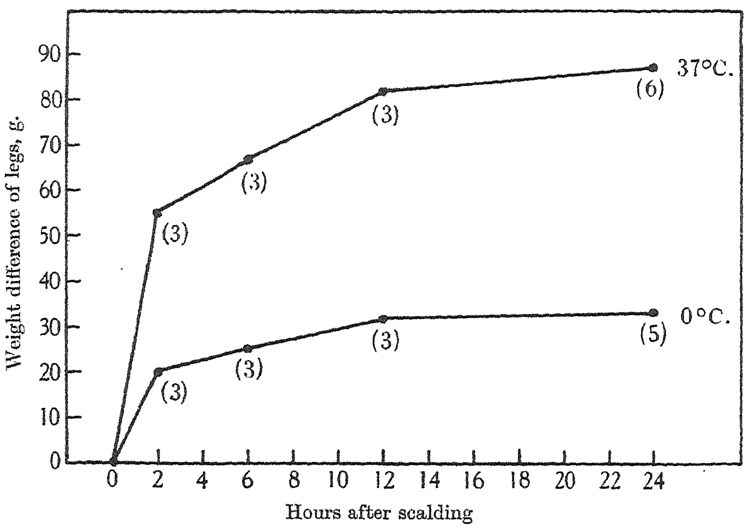
The effect of local temperature on the amount of oedema formation after scalding

The general appearance of the hindlegs of rabbits, scalded at 75° C. for 45 sec., and then kept cold (in ice-water) or warm (in a water-bath at 37° C.) can be seen in Pl. 1. In rabbit (*a*) the left hindleg has been scalded and both legs then kept in water at 0° C. for 2 hr.; in rabbit (*b*) the left hindleg has been scalded and both legs kept in water at 37° C. for 2 hr.; in rabbit (*c*) both hindlegs have been scalded, the right leg then being kept in ice-water and the left leg in water at 37° C. for 2 hr. These photographs are typical of a large series of experiments. In every case the scalded leg kept in ice-water shows little oedema formation, whereas the scalded leg kept warm at 37° C. shows immense swelling.

The actual amount of oedema formation has been measured by scalding one hindleg and then keeping both hindlegs at 0° C. or at 37° C. for the required time, when the animal was killed, the two hindlegs carefully dissected at the hip joint and weighed. The difference between the weights of the two legs has been taken as a measure of the oedema formation. Since no oozing occurs in animals, this method should give a good indication of the plasma loss from the circulation. A slight error is probably introduced by the withdrawal of tissue fluid from the undamaged tissues into the blood stream in an endeavour to compensate for the fall in plasma volume. Some of this tissue fluid will come from the undamaged leg below the knee, corresponding to the damaged counterpart, making the undamaged leg lighter and, therefore, increasing the difference in weights. The weight of the leg below the knee joint, however, is only about 80 g. in a 2300 g. rabbit, so the actual decrease in weight of the normal leg must be insignificant.

Eight groups of rabbits have been used in this experiment. In four groups the legs were kept at 0° C. after one leg had been scalded, one group for 2 hr., one for 6 hr., one for 12 hr. and one for 24 hr. Similarly, in four other groups, the legs were kept at 37° C. The average body weight in each group was 2·3 kg., and each individual rabbit was approximately 2·3 kg. At the end of the

required time for any group, the rabbits were killed and the hindlegs dissected and weighed. The average results are shown graphically in Text-fig. 1. It can clearly be seen that very much less plasma loss occurs when the burned limb is kept cool than when it is kept warm, and that in both conditions the outpouring of plasma is most rapid in the first 2 hr. and then becomes gradually slower. The plasma loss in the legs kept warm would probably continue at a greater rate but for the fact that the burned skin cannot stretch any more, and the increased tissue tension forces the oedema fluid up the leg above the upper limit of the burn. In the legs kept cold the outpouring of fluid, even



Text-fig. 1. The amount of oedema fluid in one hindleg, of the rabbit, scalded in water at 75° C. for 45 sec., when the local temperature is 0 or 37° C. The figures in brackets represent the number of rabbits in each group.

after 24 hr., is not great enough to cause tracking up the leg to the undamaged area of the thigh. Expressed as percentage of the body weight, the average loss of plasma from the one burned leg kept cold for 2, 6, 12 and 24 hr. is 0·9, 1·1, 1·3, and 1·4 respectively, whereas that from the one burned leg kept warm is 2·5, 2·9, 3·6 and 3·8 respectively. As the normal plasma volume of the rabbit is approximately 5% of the body weight (Courtice, 1943), the plasma loss from the warm leg would cause death but for the considerable withdrawal of tissue fluid from the normal tissues into the blood stream. The degree of haemoconcentration will be considered below.

In a further series of six rabbits, both hindlegs were scalded, and one leg was kept at 0° C. and the other at 37° C. for 2 hr. The average weight of the six rabbits was 2·3 kg., and the average difference of weight of the two legs

32 g., which corresponds closely with the difference in weights of the warm and cold groups at 2 hr. in the experiments represented in Text-fig. 1.

These experiments, therefore, show that if the scalded limb is kept cool immediately after scalding, the loss of plasma is very much less than when the limb is kept warm. Further experiments show that if the limb has been warmed for 2 hr. after scalding, the process of oedema formation can be slowed by then keeping the limb cool. One hindleg of each of four rabbits, each weighing 2·3 kg., was scalded. Both legs were then kept at 37° C. for 2 hr. and then in ice-water for 22 hr. The average difference in weight was 44 g. at the end of the experiment. This compares with an average difference of 87 g. had the legs been kept at 37° C. for the 24 hr. (cf. Text-fig. 1). Further evidence of the effect of cold treatment after 2 hr. at 37° C. will be given later.

The effects of local temperature have also been determined in a small series of goats. In the first experiment, both forelegs and both hindlegs of two goats anaesthetized with nembutal were immersed in drums of water at 85° C. for $2\frac{1}{2}$ min. to a depth just below the knee joint and below the elbow joint. In each animal the two comparable legs were immersed in the same drum at the same time to approximately the same depth. After scalding, the goat was stood with one foreleg and one hindleg in drums of ice-water, and the other two legs at room temperature. The goat recovered from the anaesthetic in about $\frac{1}{2}$ hr. and then stood unsupported. At the end of 6 hr. the goats were killed and the legs carefully dissected at the knee or elbow joints and weighed. The results are given in Table 1.

In two other goats the hindlegs only were scalded as previously. After scalding, one hindleg was kept in ice-water and the other in a drum of water, kept at 37° C. by a heating element and thermostat. The goats were allowed to recover from the anaesthetic. After 6 hr. the goats were killed and the legs carefully dissected and weighed. The results are given in Table 1.

TABLE 1. The effect of local temperature on the amount of oedema in legs of goats scalded at 85° C. for $2\frac{1}{2}$ min. Killed 6 hr. after scalding

Goat	Weight kg.	Hindleg wt. in g.			Foreleg wt. in g.		
		0° C.	Room temp.	Diff.	0° C.	Room temp.	Diff.
1	28	772	865	93	624	688	64
2	27	800	934	134	637	684	47
		0° C.	37° C.	Diff.			
3	25	895	1027	132			
4	27	855	1067	212			

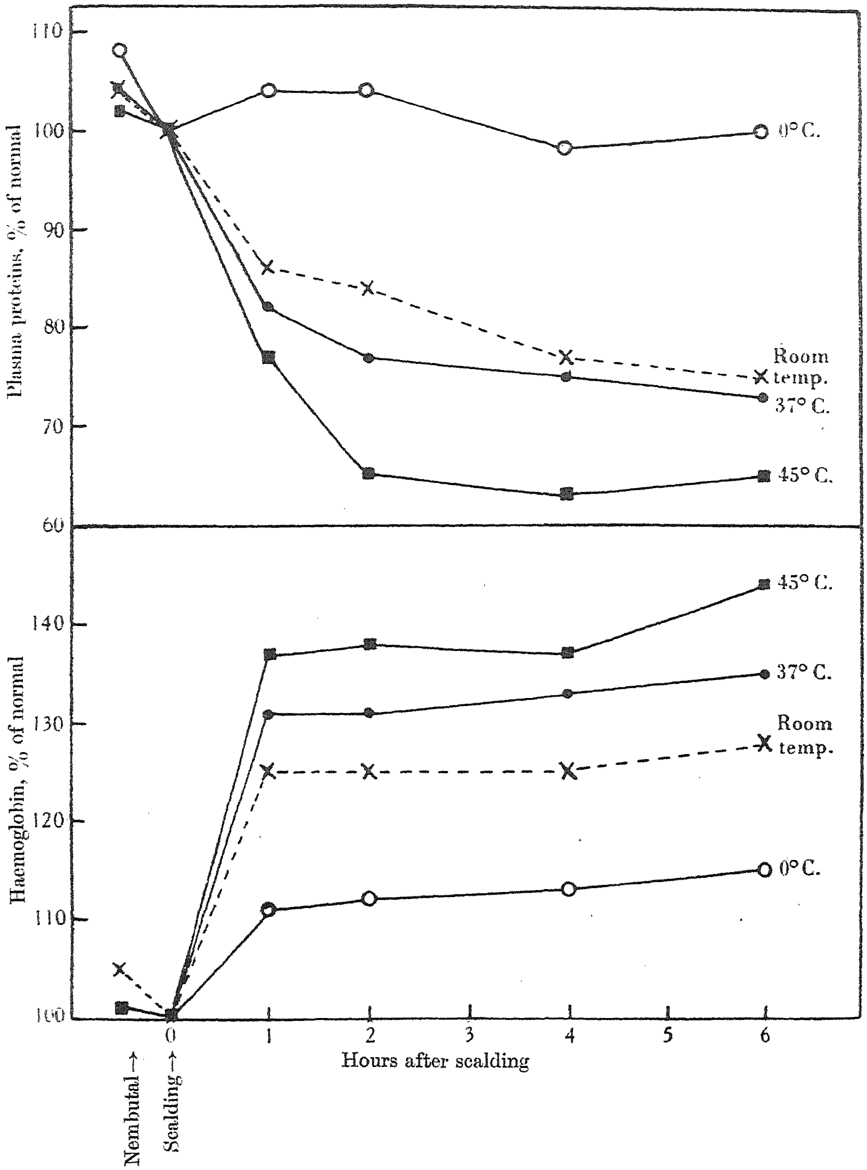
All burned legs showed oedema, but those kept in ice-water were much less oedematous than those kept at room temperature or 37° C. as the figures in Table 1 indicate. In the goat the lower part of the legs have little flesh, so the skin covers the bones fairly tightly. There is thus not room for much

oedema formation, the skin acting as a tight plaster bandage. Even so, there is a considerable difference in the oedema formation, which varies with the local temperature.

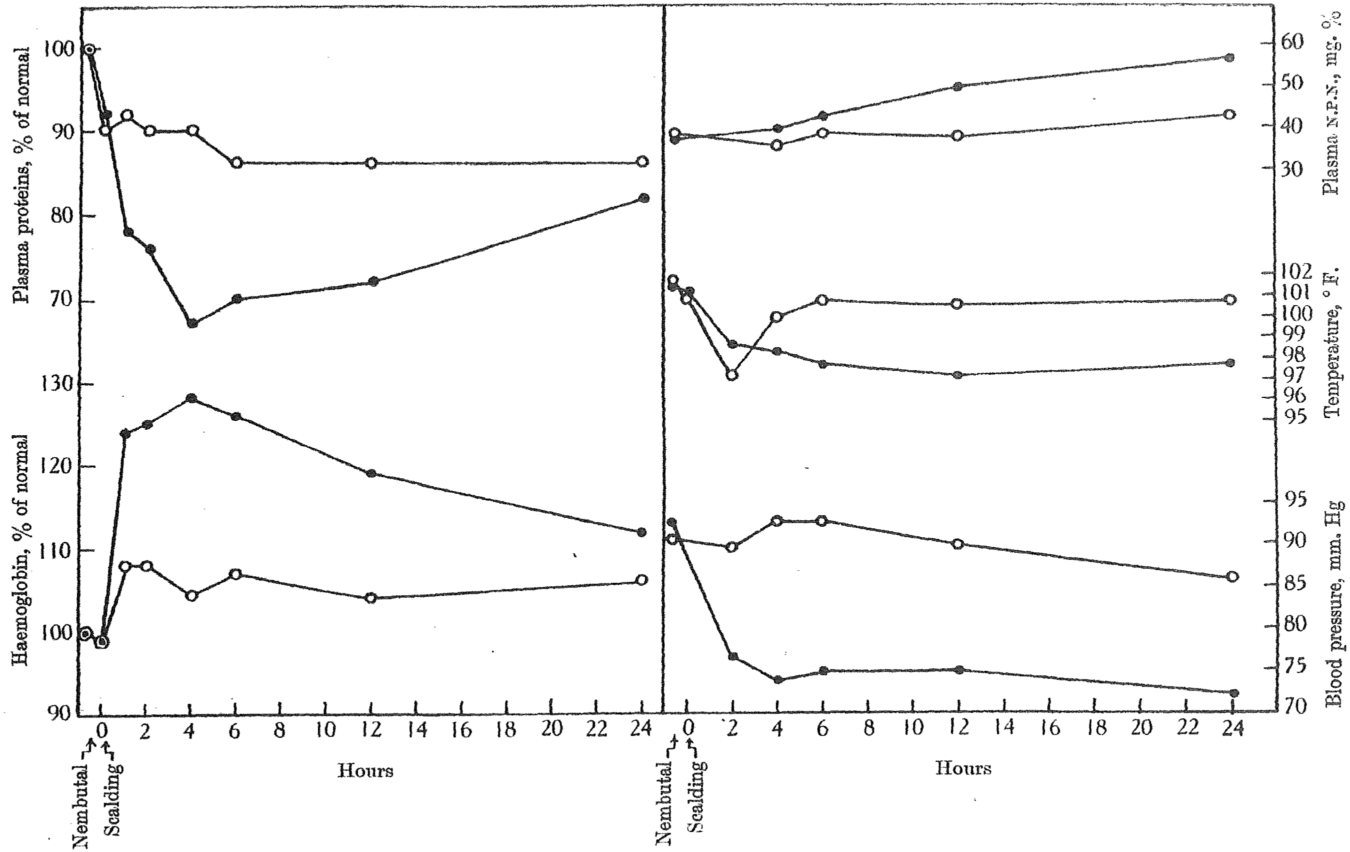
Effects of plasma loss on haemoconcentration and plasma proteins

Two series of experiments were performed in which the haemoglobin and the plasma-protein concentration were determined before and at intervals after burning. In the first series the rabbits were kept anaesthetized by repeated small injections of nembutal for 6 hr. after burning, when the experiment terminated. Both hindlegs were scalded and were then kept in a water-bath at 0, 37 or 45° C. or kept at room temperature not in a water-bath. Blood samples were taken before nembutal, after nembutal before burning, and 1, 2, 4 and 6 hr. after burning. Five animals were used in each group except the 45° C. group in which seven were used. The average results for each group are shown in Text-fig. 2. In this diagram the haemoglobin and plasma-protein concentration are expressed as a percentage of the normal value just before burning. It can be seen that the warmer the injured legs are kept, the greater the haemoconcentration and the less the plasma protein concentration. This is due to the more rapid outpouring of plasma and the greater withdrawal of tissue fluid back into the circulation, the warmer the local temperature. In the group of rabbits kept at 45° C., five animals out of seven died in 2–6 hr., so the 4 hr. point is the mean of only three animals and the 6 hr. point the mean of only two animals. It seems that in those that died, the loss of plasma was too great and too rapid to be compatible with life.

In the second series of experiments, the rabbits were allowed to recover after burning, and were examined for 24 hr. They were given cabbage to eat and water to drink. Besides the haemoglobin, plasma protein and N.P.N. concentration, the mouth temperature and blood pressure were recorded. Both hindlegs were scalded in two groups of animals. One group was then kept with the hindlegs in a bath at 0° C. and the other group in a bath at 37° C. The average results of each group of four animals are shown in Text-fig. 3. Three further animals in each group were used with similar results for the haemoglobin and plasma-protein concentration, but in these animals the blood pressure and mouth temperature were not observed. The results of the determinations on the group of rabbits whose legs have been kept at 37° C. show a rapid rise in the haemoglobin and fall in plasma-protein concentration with the rapid onset of oedema (cf. Text-fig. 1). The maximum rise in haemoglobin and fall in plasma protein occurs about 4–6 hr. after scalding. From the single burnt leg in the experiments depicted in Text-fig. 1, it would appear that the amount of fluid lost into both legs should be greater than the original plasma volume. The withdrawal of tissue fluid into the blood stream, however, prevents the haemoglobin from rising above about 130% and dilutes the plasma

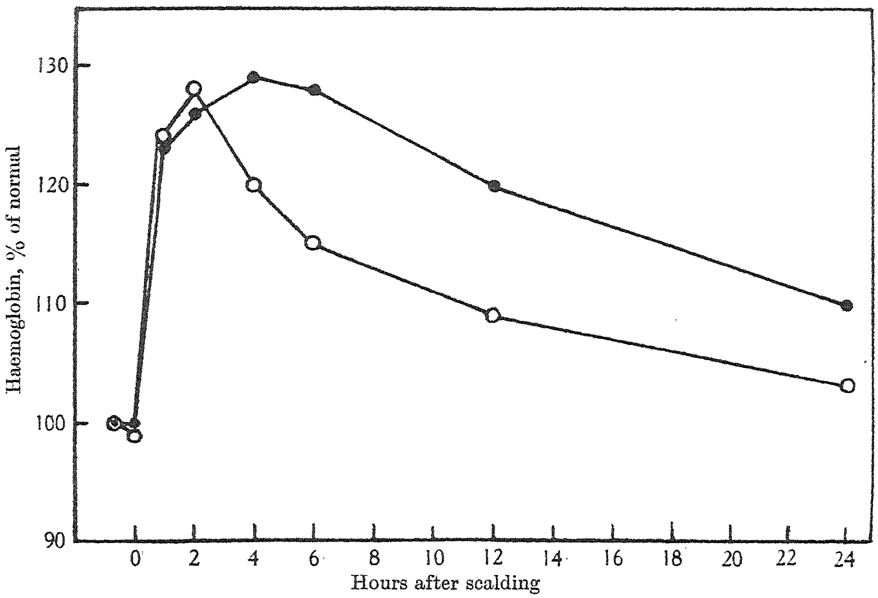


Text-fig. 2. The haemoglobin and the plasma-protein concentration represented as a percentage of the pre-scalding level when both hindlegs of rabbits were scalded. Each graph shows the average results from a group of five animals.



Text-fig. 3. The haemoglobin, plasma-protein concentration, blood pressure, mouth temperature and plasma non-protein nitrogen (N.P.N.) in rabbits. At zero, both hindlegs were scalded and the rabbits were allowed to recover from the nembutal. •——• Local temp. 37° C. Mean of four animals. o——o Local temp. 0° C. Mean of four animals.

proteins to about 70 % of their pre-burning level. After 6 hr., plasma continues to be lost locally, as seen in Text-fig. 1, but the rate of withdrawal of fluid into the circulation overtakes the outpouring of plasma into the damaged tissues, so that the haemoglobin percentage falls. The plasma-protein level also rises, due probably to regeneration or mobilization of new protein. Compared with these changes in experiments at 37° C., the changes in rabbits whose scalded legs are kept cool are very slight. The N.P.N. in the 'warm' experiments also rises during the first 24 hr., as it does in all cases of anhydraemia no matter



Text-fig. 4. Haemoglobin in two groups of rabbits after scalding both hindlegs. •——• Local temp. 37° C. for 24 hr. Mean of seven animals. o——o Local temp. 37° C. for 2 hr. and 0° C. for 22 hr. Mean of four animals.

how it is produced, whereas in the 'cold' experiments with very little anhydraemia there is practically no rise in N.P.N. The blood pressure likewise shows practically no change in the 'cold' experiments, but a considerable fall in the 'warm' experiments, with haemoconcentration. The mouth temperature in all animals burned, whether they showed haemoconcentration or not (see later experiments), fell considerably during the first 2 hr. after burning. This seems to be a nervous reaction causing an early and substantial vaso-constriction of the peripheral vessels, for during this period it is generally difficult to dilate the ear vessels by warmth, even though the blood pressure is not low. In the animals whose legs are kept cold, however, the mouth temperature then rises and remains fairly constant at nearly normal level. In the animals with legs kept warm, the mouth temperature continues to fall. This is probably due to

vaso-constriction of the peripheral vessels to compensate for the fall in plasma volume.

These experiments show that if the burned area is kept cold immediately after the injury, the blood and circulatory changes can be greatly lessened as compared with those in similarly burned animals with the legs kept warm.

It has already been seen that if the burned area is kept at 37° C. for 2 hr. and then at 0° C. for 22 hr. the oedema is much less than if the legs were kept at 37° C. for 24 hr. The effect of cold in slowing down oedema formation after 2 hr. at 37° C. can also be shown by estimating the degree of haemoconcentration. Text-fig. 4 shows the average haemoglobin changes in a group of seven rabbits with both hindlegs scalded and kept at 37° C. for 24 hr. and of a group of four rabbits similarly burned, with the hindlegs kept at 37° C. for 2 hr. and then at 0° C. for 22 hr. The haemoconcentration in both groups is almost identical in the first 2 hr. The withdrawal of tissue fluid of the second group when the legs are placed in ice-water continues as in the first group, but the outpouring of plasma into the damaged tissues is slowed, with a resultant sharp downward trend in the curve. Thus, even if cold is applied as late as 2 hr. after burning, the outpouring of plasma can be definitely slowed down.

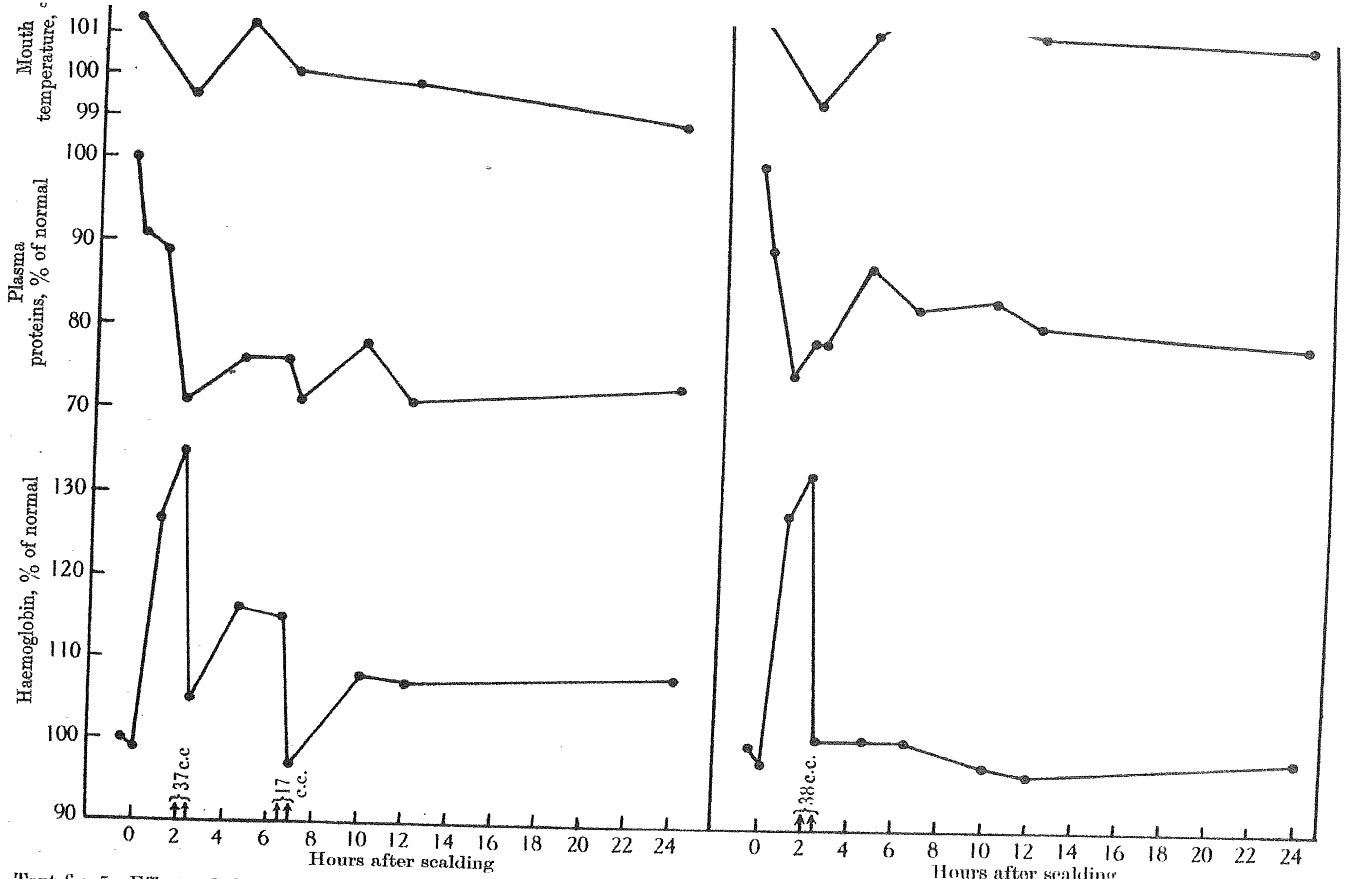
The effects of transfusion of plasma and serum

In these experiments both hindlegs of rabbits have been burnéd, and kept at 37° C. for 2 hr. At 2 hr. one group has been transferred to ice-water and transfused, while the legs of the other group have remained in the water-bath at 37° C. and the rabbits transfused. Both plasma and serum (rabbit) have been used in these transfusions.

Text-fig. 5 shows the effects of the plasma transfusions. Two hours after burning, the blood of both groups of rabbits had concentrated to about the same extent. Plasma transfusion reduced the haemoglobin to approximately the pre-burning level. The rabbits whose legs were kept warm then rapidly lost more plasma and the blood concentrated again. A further transfusion at 6 hr. again brought the haemoglobin down to normal, but further outpouring of plasma caused haemoconcentration once more. With the group of rabbits transferred to the cold water-bath, the transfusion restored the haemoglobin to normal, and this level was maintained throughout the 24 hr. After the initial fall, the plasma-protein concentration was maintained at a higher level in the cold group than in the warm group. The mouth temperature returned to normal in the cold group and remained normal after transfusion, whereas in the warm group the temperature rose after transfusion and then fell again.

Similar results have been obtained after serum transfusion.

The serum reduced the haemoglobin and increased the plasma-protein concentration and mouth temperature in the cold group, and these normal values were maintained for 24 hr. In the warm group the beneficial effect of



Text-fig. 5. Effects of plasma transfusion in rabbits after scalding both hindlegs. Left: local temp. 37° C. for 24 hr. Mean of three animals. Right: local temp. 37° C. for 2 hr. and then 0° C. for 22 hr. Mean of three animals.

the serum was transient, and as further fluid was lost into the damaged tissues, the haemoglobin rose and the plasma-protein concentration and mouth temperature fell.

These transfusion experiments show that if the affected limbs are kept cool, less plasma or serum is required to maintain the blood and circulation at a normal level, which indicates that much less leakage occurs if the burned area is kept cool than if it is kept warm.

The effects of local temperature on lymph flow from scalded paws of dogs

Lymph flow in normal legs. In these experiments the lymph duct in the hindleg or foreleg of dogs, anaesthetized with nembutal, was cannulated. Lymph was collected by massage at regular intervals, since there is no spontaneous flow. The actual rate of lymph flow in these normal limbs is, therefore, somewhat artificial and probably not very accurate, but the general trend, increase or decrease, can be determined. When the paws of a normal dog are cooled by immersion in ice-water or warmed by immersion in water at 37, 45 or 50° C., there are no very considerable changes in lymph flow. Table 2 shows the effect of cooling in a typical experiment. In this dog the

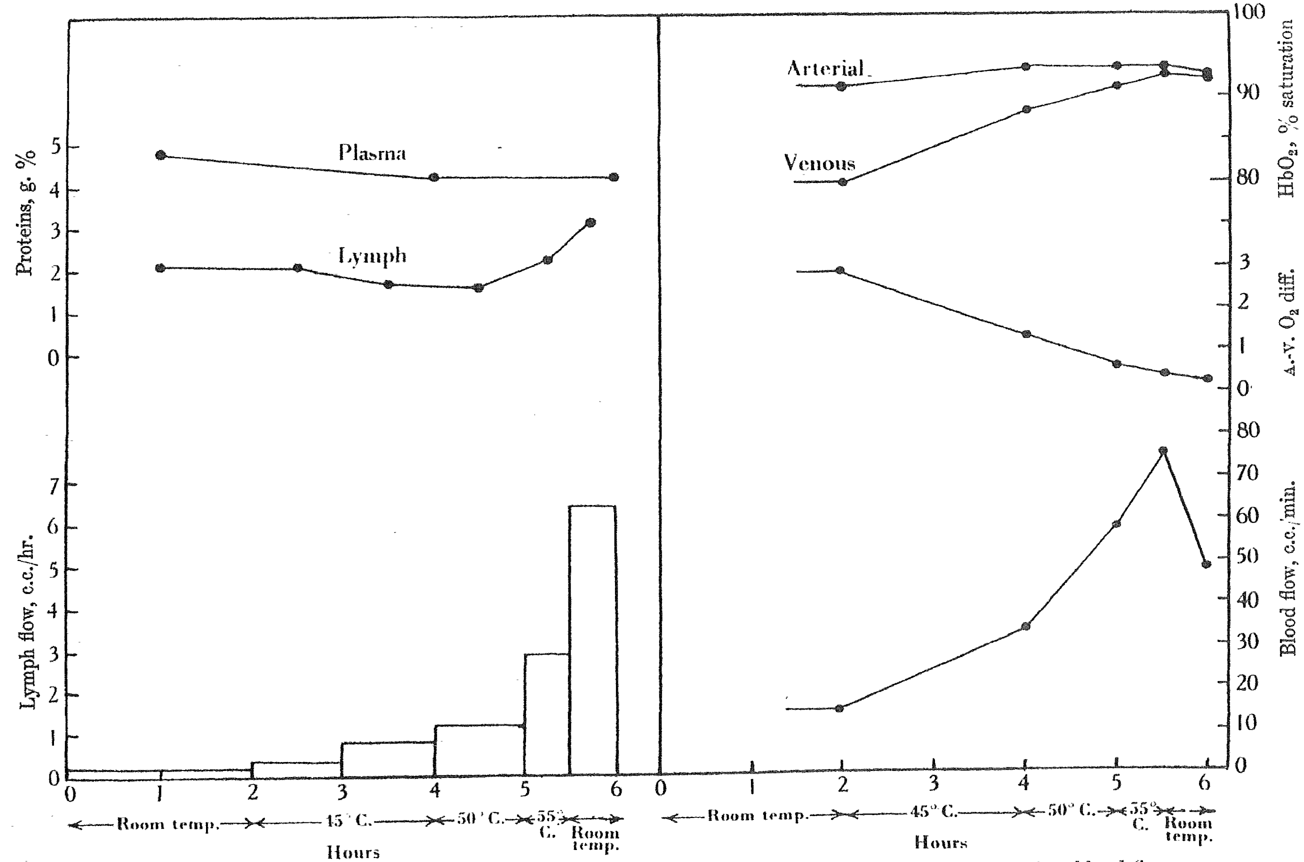
TABLE 2. The effect of cold on the lymph flow from the forepaw of a normal dog under nembutal anaesthesia

	Time hr.	Lymph flow c.c./hr.	Lymph proteins g. %
Room temp.	0–1	0·9	1·5
	1–2	1·1	1·4
Ice-water	2–3	0·7	1·4
	3–4	0·3	1·4

hindleg lymph duct was cannulated. The effect of cooling the paw was to slow down the lymph flow, without altering the protein composition of the lymph.

In Text-fig. 6 the effects of warming the forepaw on the lymph flow from the duct just above the ankle joint are shown. The lymph flow increases somewhat when the paw is immersed in water at 45° C., and still further on immersion in water at 50° C. This increased flow is accompanied by a slight fall in the protein concentration of the lymph. When the foot is then immersed in water at 55° C., the lymph flow increases abruptly, the protein concentration of the lymph rises and the paw becomes oedematous. Lassar (1889) demonstrated that when a dog's paw is immersed in water at 54° C. it becomes oedematous and the lymph flow increases.

These experiments show that temperatures as low as 0° C. and as high as 50° C. do not affect the permeability of the capillaries of the dog's paw to proteins, but at 55° C. the capillaries are damaged and the osmotic balance between the plasma and tissue fluid is upset by the increased leakage of



Text-fig. 6. The effects of warming the paw of a dog, on lymph flow, lymph and plasma proteins, blood flow, A.-V. O_2 difference and Hb saturation. Mean of two experiments.

proteins through the capillary membrane. The decrease in lymph flow on cooling may be explained by a decreased filtration of fluid through the capillaries caused by a decreased blood flow, and the increased lymph flow on warming up to 50° C. is the result of an increased formation of normal capillary filtrate following an increased blood flow. The capillary filtrate has a lower concentration of protein than tissue fluid, and so the protein concentration of tissue fluid and lymph falls somewhat as the paw is warmed, until a temperature is reached, 50–55° C. in the case of the dog's paw, where the capillary membrane is damaged. Then the capillary filtrate contains a high concentration of protein with a resultant increase in the protein concentration of tissue fluid and lymph, and a local upset in the osmotic balance.

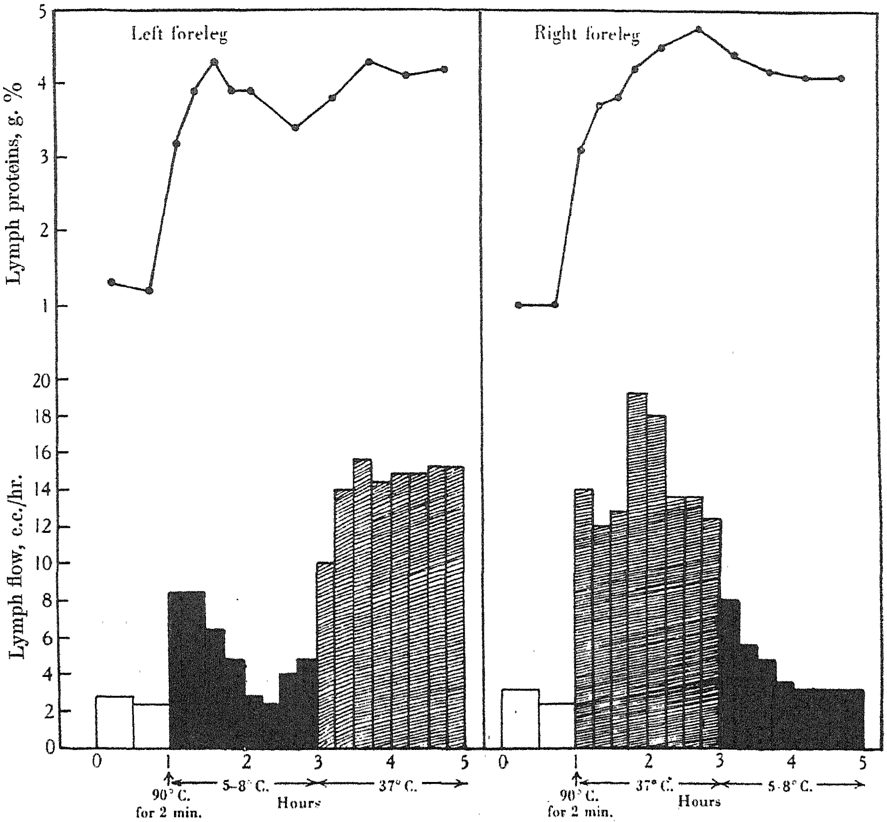
Lymph flow in scalded paws. Experiments in which the forepaws of dogs have been scalded by immersion in water at 80° C. for 2 min. or 90° C. for 2 min. and then allowed to remain at room temperature show that, after the burn, the lymph flow is greatly increased and the protein concentration of the lymph is raised to approximately the level of the plasma proteins. These results agree with the data of Field, Drinker & White (1932), Glenn, Peterson & Drinker (1942) and Glenn, Muus & Drinker (1943), and will not be given in detail.

In another series of experiments, the lymph ducts of both forelegs were cannulated, lymph collected and then both forepaws were immersed in water at 90° C. for 2 min. or 80° C. for 1½ min. One forepaw was then placed in ice-water or water at 5–8° C. and the other in water at 37° C. Lymph was collected for 2 hr., after which time the water-baths were reversed. Text-fig. 7 depicts the results of a typical experiment.

In these experiments the lymph flow before scalding was obtained by massage, but after scalding was spontaneous. In Text-fig. 7 the left forepaw was placed in water kept at 5–8° C., while the right forepaw was placed in a water-bath at 37° C. immediately after scalding. The lymph flow from both forepaws increased and was spontaneous, but the flow from the right side was very much greater than that from the left side. At the end of 2 hr. the right paw was swollen much more than the left. On reversing the water-baths, the flow from the left paw, now in water at 37° C., increased considerably, while the flow from the right paw, now in water at 5–8° C. decreased. Other experiments in which the paw was left at 0, 37 and then 45° C. after scalding show clearly that the lymph flow increases as the local temperature increases.

In these experiments the local cooling of the scalded paw decreased the swelling of that paw compared with the one kept warm, and, as a result, decreased the lymph flow. Even after the paw had been kept warm for 2 hr. after scalding, local cooling had a rapid effect in decreasing the lymph flow, which is an indication that the formation of oedema fluid was being decreased.

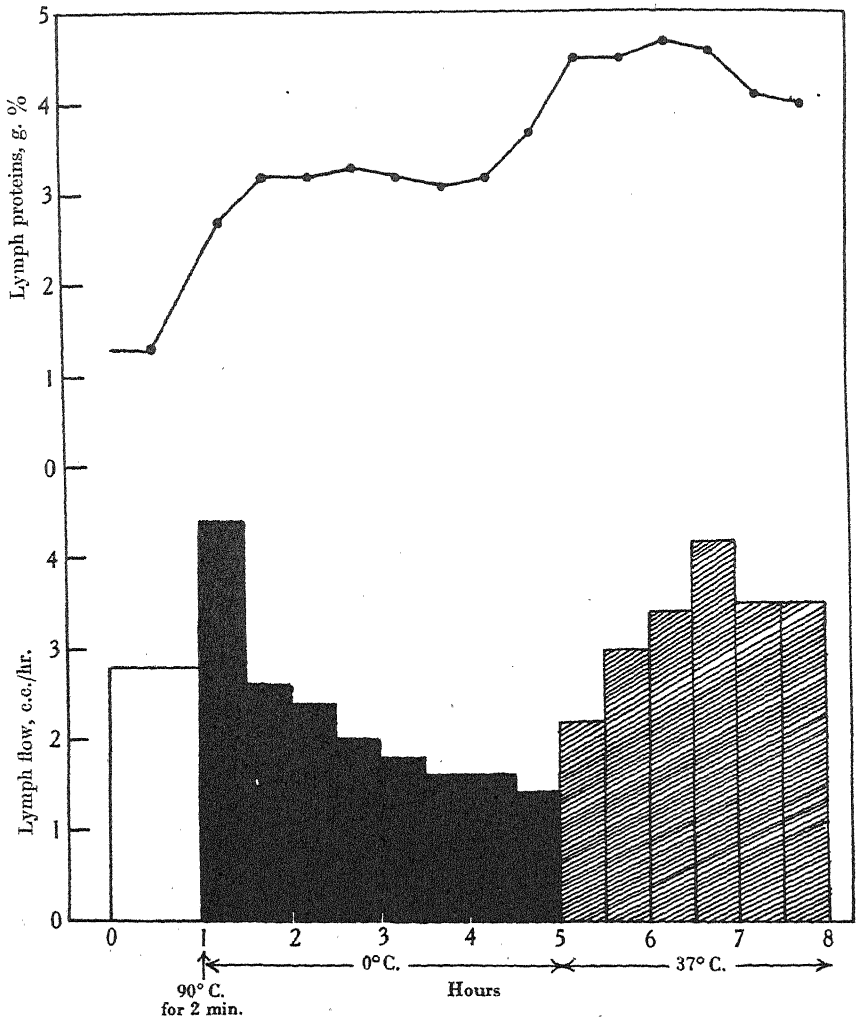
The protein concentration of the lymph in all cases rose considerably after scalding. There is an indication in all experiments that the protein level is slightly higher when the paw is kept warm than when it is kept cold. In Text-fig. 7 it can be seen that in the left paw there is a tendency for a fall after the initial sharp rise with the paw in water at 5–8° C., a fall which is reversed



Text-fig. 7. The effect of local temperature on the lymph flow from the forepaws of a dog scalded in water at 90° C. for 2 min.

when the paw is put in water at 37° C. In the right paw the protein concentration goes on rising when at 37° C., and then falls when the paw is put in ice-water. A similar effect was seen in other experiments. In Text-fig. 8 the protein concentration of the lymph after scalding, with the paw in ice-water, is fairly constant at a level of about 3·2%. When the paw is then kept at 37° C., the lymph flow increases and the protein concentration in the lymph rises to between 4·0 and 4·6%, which was the level of the plasma proteins.

It appears, therefore, that by cooling the scalded paw, not only is the production of tissue fluid slowed down, but the protein concentration of that fluid is also somewhat less.

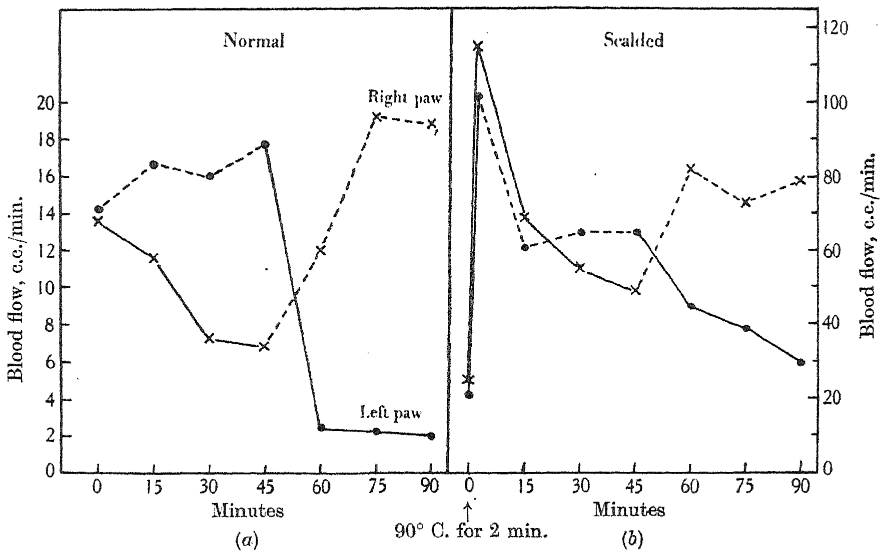


Text-fig. 8. The effect of local temperature on the lymph flow from the forepaw of a dog scalded in water at 90° C. for 2 min.

The effects of local temperature on the blood flow

The experiments so far described show that the tissue fluid formation after scalding increases with an increase in local temperature. This suggests that the blood flow through the damaged part is the determining factor. The blood flow has been determined in anaesthetized, heparinized dogs.

The effects of cooling or warming the normal paws of a dog are shown in Text-fig. 9. Immersion of the left paw in a water-bath at 37° C. and of the right paw in ice-water increased the flow in the former and decreased the flow in the latter. On reversing the water-baths, the flow in the left paw, now in ice-water, fell considerably, while the flow in the right paw, now at 37° C., rose. Text-fig. 6 also shows the effect of warming on the blood flow and the arteriovenous oxygen difference. As the blood flow increases, the arteriovenous oxygen difference decreases.



Text-fig. 9. (*a*) The effect of local temperature on the blood flow through the forepaws of a normal dog. Mean of two experiments. (*b*) The effect of local temperature on the blood flow through the forepaws of a dog after scalding in water at 90° C. for 2 min. Mean of two experiments ——— Local temp. 0° C. ---- Local temp. 37° C.

When the paws of a dog are scalded, the blood flow immediately increases greatly as shown in Text-fig. 9*b*. If one paw is then kept at 37° C. and the other at 0° C., the blood flow falls in both, but to a much lower level in the paw kept cool. When the warm paw is now placed in ice-water, there is a rapid fall in the blood flow, and when the cold paw is placed in water at 37° C. there is a sharp rise in blood flow.

Except for the experiments represented in Text-fig. 6, the determinations of blood flow have been made for only $1\frac{1}{2}$ hr. after the initial readings were obtained, with the paws kept for $\frac{3}{4}$ hr. at 37° C. and $\frac{3}{4}$ hr. at 0° C. Although the dogs were heparinized, there was always the risk of clumps of platelets forming at the ends of the cannulae, so this risk was minimized by keeping the time of the experiment relatively short. By comparison with the effects

on lymph flow, it seems that the blood flow would be still more decreased had the paw been kept longer in ice-water.

Hastings (1820), while studying problems of inflammation, showed that if the web of a frog's foot is scalded, the blood flow is accelerated and that the application of ice caused contraction of the vessels and a reduced circulation rate. (I am indebted to Prof. G. R. Cameron for this reference.)

It is, therefore, evident that cooling the limb after a burn greatly reduces the blood flow through the damaged area, as it does in a normal limb. The effect of local temperature on the oedema formation after scalding depends, therefore, on the effect on the blood flow through the injured part.

The effects of decreasing blood flow in the scalded hindlegs of rabbits by tying the femoral artery

The blood flow to the hindleg of rabbits was decreased by ligaturing the femoral artery immediately after scalding. By thus cutting down the blood supply to the injured area, the rate of oedema formation was greatly reduced. In one experiment, both hindlegs of four rabbits each weighing 2·0 kg. were scalded as previously. Immediately after scalding, the femoral artery on one side was ligatured and cut. Both hindlegs were then kept in a water-bath at 37° C. for 6 hr. The leg with the femoral artery tied showed practically no swelling while the other was greatly swollen, the mean difference in weight being 39 g.

In a second experiment, one leg was scalded and the artery tied immediately after. Both legs were then kept in a water-bath at 37° C. Four groups of rabbits, each group consisting of two animals of average weight 2·3 kg., were kept thus for 2, 6, 12 and 24 hr. respectively. The average increase in weight of the scalded leg was 8, 8, 25 and 31 g. respectively, which is very much less than the increase with the femoral artery intact (cf. Text-fig. 1).

In a third experiment, both hindlegs of three rabbits were scalded and both femoral arteries were tied immediately after scalding and the legs kept at 37° C. for 24 hr. Blood samples were taken for the estimation of haemoglobin and plasma-protein concentration; mouth temperature was also determined. The mean results are given in Table 3. These figures show very little haemoconcentration and change in plasma proteins and mouth temperature as compared with animals without ligature of the femoral artery (cf. Text-fig. 3).

In a control group of ten rabbits in which the femoral artery was ligatured and cut on one side, no ill-effects were observed in that leg, the rabbits behaving normally. Thus the collateral circulation is sufficient for the normal needs of the tissues in rabbits kept in cages at comparative rest.

The results of these experiments show that the oedema formation after burning varies with the blood flow, and support the suggestion that cold acts in decreasing the oedema by lessening the blood flow through the injured limb.

TABLE 3. The Hb percentage, plasma-protein percentage, and mouth temperature in rabbits after scalding both hindlegs and then immediately tying both femoral arteries. Mean of three animals

	Hb %	Plasma protein g. %	Mouth temp. ° F.
Before nembutal	82	5·6	101·7
After nembutal	80	4·8	—
Scalding:			
1 hr. after	83	—	98·6
2 ,,	81	5·0	99·0
4 ,,	82	4·8	100·6
6 ,,	82	4·6	101·8
12 ,,	83	4·5	101·6
24 ,,	82	4·7	102·4

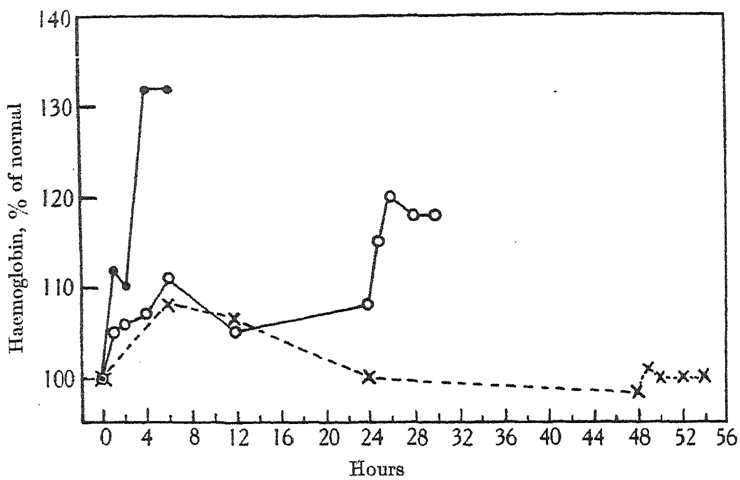
Effect of local temperature on recovery of the capillaries after a thermal burn

So far it has been shown that cold decreases the formation of oedema after thermal injury. Cold also lessens the metabolism of cells, and might, therefore, have a retarding effect on the recovery of the damaged tissue. Normally, the excess oedema formation through the injured capillaries ceases about 48 hr. after burning. Experiments were designed, therefore, to see whether, when the cold treatment ceased and the limb was then kept warm, the oedema fluid formation would suddenly increase and thereby cause latent haemoconcentration.

Both hindlegs were scalded in the usual way in three groups of rabbits. The legs were then kept in ice-water for 2 hr. in one group, 24 hr. in the second group, and 48 hr. in the third group. After being kept cold for these times the legs were immersed in a water-bath at 37° C. for 4–6 hr. Blood samples were taken and haemoglobin estimated. The haemoglobin is given as a percentage of the pre-burning level, and the results are depicted in Text-fig. 10. In the first group the haemoglobin rose to about 110% in the first 2 hr. in ice-water and then increased suddenly to 132% when kept warm. In the second group there was slight haemoconcentration during the first 24 hr., and when the legs were then kept warm, the outpouring of fluid caused a sudden increase in haemoglobin to about 120%. In the third group there was slight haemoconcentration during the first 24 hr. with a return of the haemoglobin to normal by 48 hr. The effect of warming the legs in this group was to cause only a very small increase in the haemoglobin.

Thus, the longer the injured part is kept cool up to 48 hr., the less will be the haemoconcentration when this part is warmed. The fact that after 48 hr. there is but little increase in haemoglobin when the legs are warmed does not necessarily mean that the capillary damage has been completely repaired by then, but it appears that after this time there will be no sudden increase in oedema fluid formation on warming, likely to cause any appreciable circulatory disturbance.

In another group of experiments to find whether cold for 48 hr. retards subsequent healing, small burns were made on the outer side of each hindleg in three rabbits. The burns were made by a closed, hollow copper cylinder, 2 in. long and $\frac{1}{2}$ in. in diameter. Water at 75° C. flowed continuously from a reservoir through an inlet near the bottom and out through an outlet near the top. This cylinder was held on the shaved skin of the rabbit's leg for 45 sec., producing a circular burn, $\frac{1}{2}$ in. in diameter. Two such burns were made on each leg. One leg of each rabbit was then kept in ice-water for 48 hr. and the other leg in a water-bath at 37° C. for 48 hr. After that time, the rabbits were



Text-fig. 10. The haemoglobin in three groups of rabbits after scalding both hindlegs. •——• Local temp. 0° C. for 2 hr. and then 37° C. for 4 hr. o——o Local temp. 0° C. for 24 hr. and then 37° C. for 6 hr. ×----× Local temp. 0° C. for 48 hr. and then 37° C. for 6 hr.

allowed to remain in their cages at room temperature. The burns were found to heal at about the same rate on each leg, the mean healing time of the six burns kept cold being 17·1 days and that for the six kept warm 17·5 days.

In a further group of six rabbits, one burn was made on each leg. The femoral artery on one side was then immediately ligatured and cut. The burns healed at exactly the same time on both sides, the mean time being 15·3 days.

It is evident, therefore, that by decreasing the blood-flow to a limb by the application of cold or by ligaturing the main arterial supply, the healing time of a small burn is not affected. The cold applied to local burned areas should thus not have a deleterious effect on the subsequent course of the burn, but only a beneficial effect in lessening and slowing the degree of local fluid loss.

Comparison of the effects of cold and of pressure bandages

To compare the effects of close-fitting plaster bandages with those of cold on the local fluid loss, one hindleg of each rabbit of four groups (four in each group) was scalded as above. Immediately afterwards, a plaster of Paris bandage was applied up to a level above that of the burn. Both normal and burned legs were then kept in a water-bath at 37° C. for 2, 6, 12 or 24 hr., when the animal was killed and the hindlegs dissected and weighed. The average amount of fluid loss in each of these four groups (each group averaged 2·3 kg. body weight) was 17, 21, 23 and 22 g. respectively. These figures are of the same order, but slightly less than those obtained for cold (cf. Text-fig. 1). The amount of oedema depends largely on how tightly the bandage is applied. In these experiments the bandages were very closely applied.

Discussion

The local loss of fluid

Provided a burn is not severe enough to cause almost complete coagulation and necrosis of the injured part, the local loss of fluid plays a considerable part in the subsequent course of the burn, especially in the early stages. The burns employed in this investigation have been of sufficient severity to damage the local capillaries, but not to produce coagulation of the limb. A rabbit's ear, for example, will shrivel up if placed in water at 90° C. for 2 min., whereas a dog's paw will not. Prinzmetal, Bergman & Hechter (1944) have also shown in rats that a leg immersed in water at 75° C. for 10 sec. will become very oedematous, whereas a leg immersed in water at 100° C. for 2–3 min. shows little oedema. Thus burns of different severity have been used here for different animals, since only one factor, fluid loss, has been studied.

In the present series of experiments with rabbits, it has been shown that when only one hindleg is scalded to a level just below the knee joint and then kept warm (37° C.), the local fluid loss is 2·5% of the body weight after 2 hr., 2·9% after 6 hr., 3·6% after 12 hr., and 3·8% after 24 hr. Since the fluid lost from the circulation closely resembles plasma in composition, these figures represent a very considerable and sudden plasma loss, being more than half the original plasma volume (Courtice, 1943). Even though modern treatment by plasma transfusions can replace plasma loss, it is obviously important to decrease and slow down the amount of oedema formation as much as possible.

It is the effect of this fluid loss on the plasma volume which is of primary importance. Hence the rate of fluid loss is as much a determining factor as the actual amount. The fall in plasma volume is determined by the balance between the local plasma loss and the tissue fluid withdrawal from the undamaged tissues into the blood stream. In haemoconcentration due to thermal

burns and other agents (Courtice, 1943; Cameron *et al.* 1945; Cameron & Courtice, unpublished results), the haemoglobin percentage reflects fairly accurately the changes in plasma volume. Thus in the experiments described above, during the first 6 hr. after the burn in rabbits with both hindlegs scalded below the knee and then kept at 37° C., the fluid loss is greater than the withdrawal of tissue fluid into the circulation, although this latter must be considerable, for the loss of plasma in 2 hr. is about equal to the original plasma volume, and yet the haemoglobin is increased to only 130% of the normal value. From about 6 hr. onwards, the outpouring of plasma continues, but at a slower rate (Text-fig. 1), and the withdrawal of tissue fluid into the circulation together with fluids by mouth must exceed the fluid loss, for the haemoconcentration decreases. The fall in the plasma-protein concentration is also an indication of the fluid loss and of the degree of withdrawal of tissue fluid into the blood stream. This fall in the plasma protein and the rise in the haemoglobin percentage run parallel with the amount and rate of fluid loss, which in turn vary with the local temperature.

A slowing effect of cold on oedema formation after traumatic injury has been described by Blalock (1942). He investigated the effects of local application of heat and cold to the traumatized limbs of anaesthetized dogs. The survival time of dogs whose injured limbs were kept cold was twice as long as those whose limbs were kept warm. These experiments suggested that the cold lessened the rate of oedema formation, but that the trauma was severe enough to produce death in spite of this slowing.

Rose (1936) has used cold-water treatment in human burns, by immersing the whole body in a bath as well as swabbing the local burned areas with cold water. He maintains that especially in the initial phase much benefit is derived from this treatment, relief from pain being marked.

The lymph and blood flow

Evidence of the mechanism in the slowing of oedema formation when the injured limb is cooled is given in the experiments on lymph and blood flow in the dog. In a normal dog's paw, the lymph and blood flow are slowed by cooling in ice-water and increased by immersion in water up to 50° C. without any apparent injury to the capillaries. The lymph-flow changes are not very great until a temperature of about 55° C. is reached, when the capillary permeability is increased and the protein concentration of the lymph increases.

After a thermal burn in which the capillaries become freely permeable to the plasma proteins, the lymph flow varies with the rate of tissue fluid formation. As the protein concentration in the tissue fluid becomes approximately the same as that in the plasma, the osmotic balance at the capillary membrane is upset, so that there is probably little if any reabsorption of capillary filtrate into the blood stream. The lymphatics, therefore, carry away the excess tissue

fluid, and the faster the tissue fluid is formed the greater is the lymph flow. The effect of local temperature on the tissue fluid formation can, therefore, be gauged by the lymph flow, at least in the early stages before much clotting of the fluid occurs. Cooling of the dog's scalded paw results in a lessening of the lymph flow as compared with warming the paw, which indicates a lessening of the filtration through the capillaries with a resultant decrease in tissue fluid formation.

The primary cause of the effect of local temperature on the fluid loss seems to be the blood flow. In a normal limb, the blood flow decreases on cooling and increases on warming, but these changes in blood flow do not cause very appreciable alterations in tissue fluid nor in lymph flow. The fluid balance seems, therefore, to be controlled mainly at the blood capillary. When a limb is scalded, however, the osmotic balance is upset with little or no capillary reabsorption. The effect of alterations of blood flow on tissue fluid formation are then considerable, as has been seen above. The only removal of the fluid is by the lymphatics, which cannot deal efficiently with such a rapid outpouring of fluid. Thus, by keeping a good blood flow through the injured part by warmth, the tissue fluid formation is considerable, whereas by decreasing the blood flow and probably also the capillary pressure, by cooling, the amount of oedema formation is lessened.

It has also been noted that the lymph-protein concentration is somewhat less when the scalded limb is kept cold than when it is warm. It may be that the cold causes contraction of the capillaries which renders them less permeable to the proteins, whereas warmth causes dilatation which stretches the walls of the capillaries and makes them more permeable. Another factor which may be involved is the capillary pressure, which is probably lower in the cold limb with a decreased blood flow and presumably contracted arterioles.

It is evident from the results described that the local application of cold considerably lessens the oedema formation after scalds in rabbits, goats and dogs. From the results of small burns in rabbits and of the effects of sudden warming after keeping the injured legs cold, it appears that the application of cold for 48 hr. does not have any appreciable slowing effect on the ultimate recovery of the burn. Thus, so long as the general body temperature is maintained, the local application of cold should lessen the fluid loss and so lessen the tendency to circulatory collapse, especially if applied in the early stages. From the work of other investigators, it appears that cold might decrease pain (Rose, 1936; Smith, 1942) and bacterial infection (Crossman, Ruggiero, Hurley & Allen, 1942; Mock & Mock, 1943), two other important features in burns.

Summary

1. The effects of local temperature on the fluid loss after thermal burns in rabbits, goats and dogs have been investigated.

2. The legs of these animals have been scalded by immersion in hot water—rabbits at 75° C. for 45 sec., goats at 85° C. for $2\frac{1}{2}$ min., and dogs at 80° C. for $1\frac{1}{2}$ min. and 90° C. for 2 min. After scalding, the injured parts have been kept at a constant temperature by immersion in water at 0, 37 or 45° C.

3. The amount of local fluid loss when one leg of a rabbit is scalded is nearly three times as great when the leg is afterwards kept at 37° C. as when it is kept at 0° C. Not only is the rate of oedema formation decreased, but, up to 24 hr., the actual amount of oedema is also decreased.

4. In rabbits and goats with both hindlegs scalded, one leg kept in ice-water always showed much less oedema than the other kept in water at 37° C.

5. The effect of the local temperature after scalding on haemoglobin content, plasma-protein concentration, blood pressure, plasma non-protein nitrogen and mouth temperature has been determined in rabbits. The degree of haemoconcentration runs parallel with the degree of plasma loss, which in turn varies with the local temperature. A burn which might cause death from circulatory collapse if the injured part were kept very warm might not if the part were kept cold.

6. The application of cold after the scalded legs have been kept warm for 2 hr. rapidly causes a decrease in the rate and amount of fluid loss and also in haemoconcentration.

7. Plasma and serum transfusions are more effective in reducing haemoconcentration when the injured part is kept at 0° C. compared with 37° C.

8. The lymph flow from the scalded paws of anaesthetized dogs is much less when the injured part is kept cold than when it is kept warm. The protein concentration of the lymph is also slightly less when the paw is cold than when it is warm.

9. The blood flow through the scalded paw of a dog is greatly decreased by the application of cold as compared with warmth.

10. The plasma loss in a scalded limb appears to depend upon the blood flow which can be altered by altering the local temperature. In experiments in which the blood flow in the scalded legs of rabbits was decreased by tying the femoral artery, the plasma loss and haemoconcentration were also greatly reduced.

11. A decreased blood flow, caused by ice-water for 48 hr. or by ligature of the femoral artery, had no effect on the subsequent recovery of small burns in rabbits.

12. The effects of cold are comparable to those of pressure bandages when applied to the scalded legs of rabbits.

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THE NORMAL COURSE OF SEPARATION OF THE PUBES IN PREGNANT MICE

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(*Received* 31 *July* 1945)

Contrary to the statement by Burrows (1935), the symphysis pubis of the mouse is replaced during pregnancy by a ligament, and, at parturition, the pubic bones are separated by a distance of some millimetres (Gardner, 1936). The separation, which occurs chiefly in the last week of pregnancy, depends on the simultaneous presence of placentae and ovaries, and is independent of the presence of the pituitary and of foetuses (Newton & Lits, 1938; Newton & Beck, 1939).

It should be possible to reproduce the physiological separation in the non-pregnant oöphorectomized animal when the factors determining it are fully understood. A clear idea of its normal course is a necessary preliminary, and the object of this investigation is to supply this need.

Methods

With the aid of a dental X-ray machine, photographs have been taken of the symphysis pubis of the mouse during pregnancy. Fig. 1 shows the method diagrammatically. The X-rays are directed at 45° to the horizontal in the sagittal plane of the animal on to its perineum. The photographic film is placed beneath the animal's pelvis and the pubic region is pressed down on to the film so that the interpubic interval, as measured on the negative, is as nearly as possible identical with

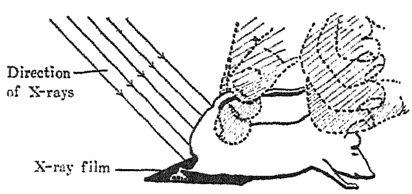

Fig. 1.

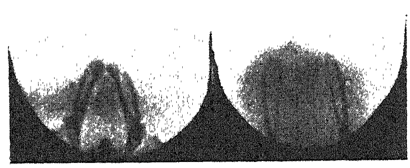

Fig. 2. A. Pelvis of mouse 441218E, day 6.
B. Pelvis of mouse 450109C, day 20.

that which would have been obtained by direct measurement. It is necessary to hold the tail of the mouse so as to tilt the pubis backwards out of the way of other bony structures. A lead screen is used to protect the hand of the operator. The exposure, determined by trial and error, was 0·85 sec. with the anti-cathode 19–20 cm. from the perineum of the mouse. Mice held in the way described remain perfectly still and do not need anaesthetizing. A typical photograph is shown in Fig. 2. The shadow of the pelvis as a whole is distorted, but it gives a satisfactory measurement of the interpubic gap. Table 1 compares live X-ray and post-mortem direct measurements made on the same day on thirty mice. In only two is there a discrepancy of more than 0·5 mm., and

whereas this may be a large percentage error when the separation is small, it is impossible, except where the edges of the bone are very distinct, to make direct measurements in the dead mouse more accurately than to the nearest 0·5 mm. Measurements on the photographs are made with the aid of dividers, and the fractions of millimetres are estimates. Error from parallax is negligible.

TABLE 1. A comparison of the length in millimetres of the interpubic gap as measured in thirty mice (*a*) during life, using X-ray photography, (*b*) after killing, on the actual pelvis. The measurements were made within a few hours of each other

Serial no.	(*a*)	(*b*)	Serial no.	(*a*)	(*b*)
X5	5·4	5·5	75	1·0	1·0
X2	4·0	4·0	81	1·0	1·5
450325B	3·5	4·0	84	1·0	1·0
X3	3·4	4·0	88	1·0	1·0
X	2·2	2·0	89	0·8	1·0
76	2·0	2·0	80	0·7	1·0
79	2·0	2·0	86	0·7	0·5
87	2·0	1·5	65	0·5	0
E78	1·8	2·0	66	0·5	Mobile
40	1·7	1·0	77	0·5	0·5
450325A	1·5	1·5	82	0·5	0·5
39	1·5	1·5	68	0·25	Mobile
70	1·5	1·5	69	0·25	Mobile
41	1·0	0·5	E38	0·25	0·5
67	1·0	0·5	X4	0	0

The recorded length is always the shortest distance between the bones. This does not always give a true idea of the size of the ligament, because the gap appears to be produced partly by a rotation of the two halves of the pelvis so that the caudal part of the symphysis is spread out. Ligament formation may take place extensively in this part of the gap before much separation of the nearest points of the bones at the cephalic end has occurred. On account of the shape of the ligament and the direction of its fibres, the 'length' of the ligament and the 'width' of the gap refer to the same measurement.

In normal non-pregnant mice the separation between the bones is sometimes measurable and sometimes only just visible. At this stage of the investigation it is inadvisable to adopt any rigid conventions, so that 'closed' symphyses are recorded as being separated sometimes by 0·25 and sometimes by 0 mm., according to the appearance on the film. In Table 2 the majority of gaps on the 6th day of pregnancy measure 0·25 mm., whereas, in another investigation on oöphorectomized non-pregnant mice, the gaps are only just visible and are recorded as zero. It is possible, therefore, that this small separation on the 6th day may have some significance, though it remains unchanged for a further week.

Mated mice were examined every morning for seminal plugs, and, when these were found, the midnight following was assumed to mark the end of the 1st day of pregnancy. This is the convention adopted by Marrian & Newton (1935), Newton (1935), where it is incorrectly stated, and Brooksby & Newton (1936). Thus, procedures during the working hours of the nth calendar day after that on which the plug was found are carried out during the $(n+1)$th day of pregnancy. In Newton & Lits (1938), Newton & Beck (1939), Newton & Richardson (1940) and Deanesly & Newton (1940), the day of finding the plug is regarded as zero, and 'the nth day of pregnancy' means 'the working hours of the nth day after that on which the plug was found'. We feel that in following a process such as separation of the symphysis, it is advisable to return to the original and more accurate terminology.

RESULTS

The state of the symphysis was recorded during the 6th day of pregnancy and then on the 13th, 15th, 17th, 19th, 20th and 21st days. One or more of these routine measurements was occasionally missed, and some measurements were

TABLE 2. The width of the interpubic gap in millimetres at different periods during the first pregnancy in seventeen mice

Serial no.	Day of pregnancy 6	13	15	16	17	19	20	21	23	No. of young
441209A	0·25	0·5	2·3	—	5·0	7·2	—	—	—	5
18D	0·25	0·25	1·0	2·5	3·8	5·7	3·0	2·2	2·0	7
11F	0·25	0·5	1·0	—	3·0	5·5	—	2·0	1·3	—
16B	0·25	0·25	1·5	—	3·0	5·5	2·8	1·8	1·3	7
18C	0·25	0·5	—	2·0	3·2	5·5	3·5	2·5	1·5	3
08C	0·25	—	1·4	—	—	5·4	—	2·5	2·0	—
08B	0·25	0·25	1·0	—	3·0	5·0	—	2·5	2·0	—
08E	0·25	0·25	1·0	—	3·6	5·0	—	—	0·8	—
09B	0·25	0·25	1·0	—	3·2	5·0	—	2·8	1·7	3
18B	0·25	—	0·8	2·2	—	5·0	4·0	2·8	1·4	4
18E*	0·25	0·25	0·7	2·0	3·0	4·7	5·0	3·0	2·0	2
07A	0	0	1·0	—	2·5	4·2	—	—	1·8	—
09C	0·25	0·5	1·0	—	3·0	4·0	—	1·0	—	—
18A*	0·25	0·25	—	2·5	—	3·8	4·7	3·0	1·4	5
08A	0·25	0·25	—	0·8	1·5	3·0	—	1·5	1·0	—
11D	0·25	0·25	0·8	—	2·8	—	—	1·5	1·0	—
18G	0·25	0·25	—	2·2	4·2	—	4·2	2·5	1·9	7
Average	—	—	1·1	2·2	3·2	5·0	3·9	2·3	1·4	

* These mice littered on the night of the 20th–21st day. The remainder littered during the preceding night.

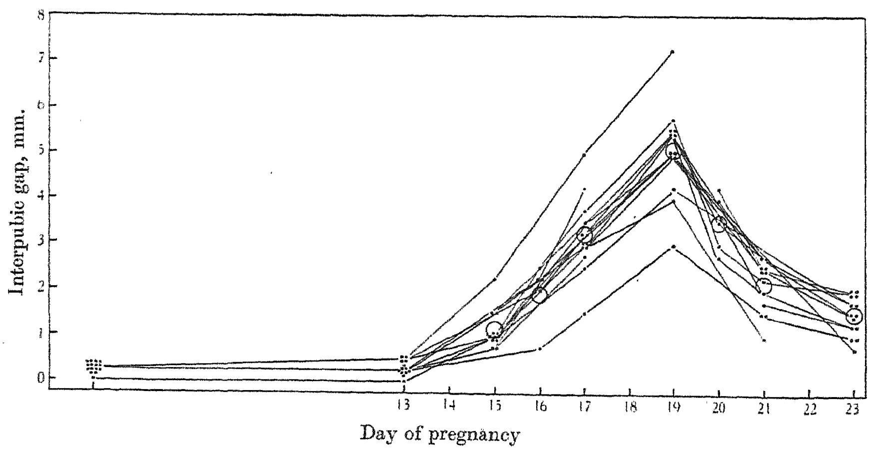

Fig. 3. The course of separation of the symphysis pubis during the first pregnancy. The data of Table 2 plotted to show the relation of the average curve to the individual curves. The two mice which littered late are excluded both individually and from the averages. Since parts of some of the curves coincide, a separate dot is plotted for each observation, at the expense of a little displacement where measurements coincide. The averages of the daily measurements after the 13th day lie at the centres of the circles.

interpolated on other days. Results for previously virgin mice are shown in Table 2 and Fig. 3.

Although the separation in each mouse follows a smooth course, there is much individual variation in its extent. Table 3 records direct measurements

TABLE 3. Findings in twenty-one mice killed on the 18th day of the 1st pregnancy

Serial no.	Interpubic gap mm.	No. of foetuses	Total wt. of foetuses g.	Wt. of mouse g.
109 A	6·5	9	9·8	29·3
417	5·0	7	3·4	32·4
201 B	4·0	6	5·7	29·5
320 A	4·0	8	6·1	29·9
322 D	4·0	5	3·5	31·3
109 B	3·5	7	5·15	30·4
321 B	3·5	6	4·2	27·8
322 A	3·5	9	6·0	31·9
130 B	3·0	6	4·0	29·0
201 A	3·0	5	3·0	26·15
320 B	3·0	5	4·4	33·7
321 A	3·0	8	4·6	29·1
321 D	3·0	4	3·8	29·6
322 B	3·0	10	6·5	29·0
322 C	3·0	9	5·9	36·2
202 B	2·5	2*	1·4	27·3
322 E	2·5	6	3·9	26·5
212 A	2·0	2	1·6	29·2
212 B	1·5	1	0·6	33·2
321 C	1·5	5	3·0	24·8
322 F	1·0	2	1·15	26·6
Average	3·1	—	—	—

* Five out of seven foetuses removed surgically on the 12th day.

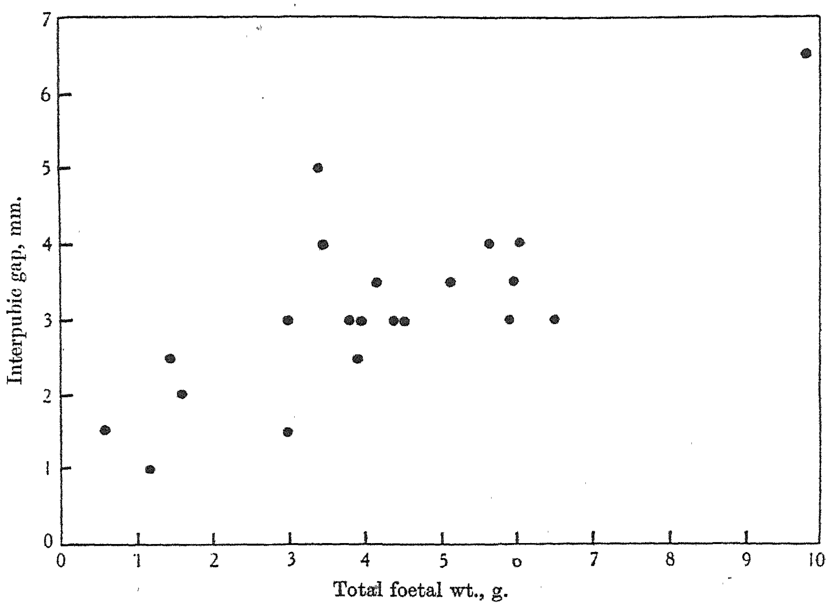

Fig. 4. To show a possible relationship between the length of the interpubic gap and the total weight of foetuses in twenty-one mice killed on the 18th day of the 1st pregnancy.

made on twenty-one mice killed on the 18th day of pregnancy. There was no obvious relationship between the width of the interpubic gap and either the body weight of the mice or the number of foetuses. Fig. 4 suggests a relation between the extent of the gap and the total foetal weight, but the two can obviously vary independently. Comparison of Tables 2 and 3 shows the existence of an unknown factor. The mice belonged to different colonies reared in different laboratories, but derived from the same stock. The gap on the 17th day in the mice of Table 2 is almost identical with that on the 18th day in the

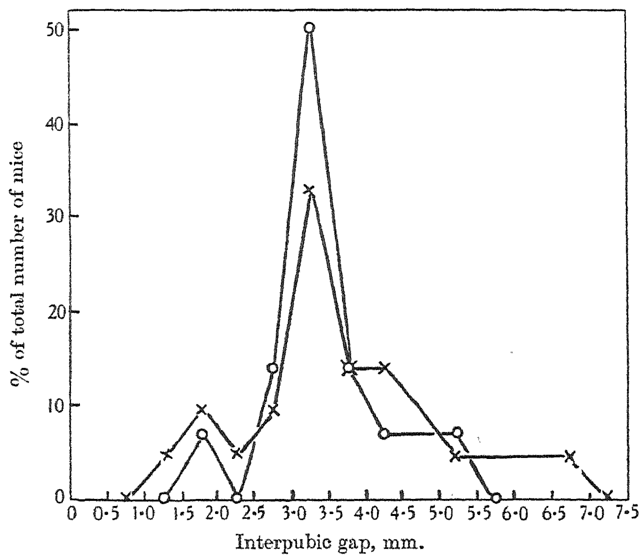

Fig. 5. Frequency distribution of widths of interpubic gap in two batches of mice of the same stock kept under different conditions. ×——×, 21 mice killed on the 18th day. o——o, 14 mice X-rayed on the 17th day.

mice of Table 3 (Fig. 5). The ages at which the mice became pregnant may have had an influence, but the ages were not recorded, and an unidentified environmental factor is an obvious possibility.

There was a rapid reduction in the length of the ligament immediately after parturition, but, approximately 3 weeks later, twelve mice had ligaments respectively of 0·8, 1·4, 0·9, 1·0, 1·0, $<1·0$, 0·8, 0·7, 1·2, 1·0, 1·2, 1·8 mm. (the last five measurements were made on the 6th day of a second pregnancy, but their identity with those made on the 13th day (Table 4) shows that they may be accepted). The average is just over 1·0 mm. There is, therefore, some substance in Gardner's statement that the changes are irreversible, but 'not completely reversible', would be more correct. However, months after the 3rd, 4th and 5th pregnancies, he found ligaments from 5 to 6·5 mm. in length,

TABLE 4. The width of the interpubic gap in millimetres at different periods during the second pregnancy in six mice

	Day of pregnancy					
Serial no.	6	13	19	20	25/26	No. of young
450202	0·7	0·9	5·0	5·0	1·4	5
450203 C	1·2	1·2	7·0	4·3	1·1	4
450206 E	1·0	1·0	4·9	—	—	6
450206 F	1·2	1·4	6·8*	—	3·0	8
450209 D	1·8	—	5·8	6·9	2·8	3
450210	—	1·2	5·2	2·9	1·0	6

* At least. Only one pubic bone appeared on the photograph.

and our records of 2nd pregnancies (Table 4) suggest that the changes may become progressively greater with successive pregnancies, in spite of two of the ligaments having returned to their original length by the 25th day.

DISCUSSION

The purpose of this work was to establish a standard of comparison, and although we have encountered variability to which no obvious factor of correction can be applied, it is clear that an average rate of expansion of the ligament of 1 mm. a day is a useful guide in any attempt to produce pubic separation artificially. (Unrecorded observations suggest that some strains of mice undergo greater change than others.)

The most constant and striking findings are the commencement of the pubic separation between the 13th and 15th days of pregnancy, its prompt regression after parturition, and its partial persistence. It may be significant, or a mere coincidence, that the foetal placental circulation becomes established at about the 13th day (Jenkinson, 1902). A large number of observations made from time to time reveal that on the 13th day of pregnancy the symphysis is always mobile. We have no evidence whether this is the first stage of that change which proceeds so quickly between the 15th and 20th days, or whether it is a phase of some preliminary and less dramatic process. The persistence of a short residual ligament, our records of second pregnancies, and both Gardner's recorded and our own unrecorded observations of long ligaments in multiparous mice, all suggest a slow irreversible process of separation during pregnancy on which the rapid reversible process of the last 5 days is superimposed. This is a purely speculative idea, but important to bear in mind in view of Burrows's (1935) and Gardner's (1936) demonstration that oestrogen will produce an interpubic ligament after some weeks of treatment with fairly large doses, though it will not bring about the rapid change observed in pregnancy.

The bearing on this problem of the work of Abramowitz, Money, Zarrow, Talmage, Kleinholz & Hisaw (1944) on relaxin will be discussed in a subsequent paper.

Summary

1. A method of measuring the width of the interpubic gap, using X-rays, in the unanaesthetized pregnant mouse is described.

2. Separation of the symphysis pubis during the first pregnancy is negligible until the 13th day, after which it proceeds at the rate of about 1 mm. per day until parturition, which usually occurs on the 19th or 20th day. Direct observation shows the symphysis to be mobile on the 13th day.

3. For 2 days after parturition the symphysis closes more rapidly than it opened. Thereafter it closes more slowly and a small permanent separation remains.

4. The separation is greater in the 2nd pregnancy.

Our thanks are due to the Medical Research Council by whom the major expenses of this research have been defrayed, and to Mr C. A. Evans for much assistance at University College, London, where some of the work was done. We are greatly indebted to Prof. H. H. Stones of Liverpool for X-ray facilities in the School of Dental Surgery.

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THE RENAL ACTION OF POSTERIOR PITUITARY EXTRACT AND ITS FRACTIONS AS ANALYSED BY CLEARANCE EXPERIMENTS ON RATS

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Renal clearance estimations, after injection of posterior pituitary extracts, have been performed by Poulsson (1930) with creatinine on dogs, by Burgess, Harvey & Marshall (1933) with sucrose and xylose on man and dogs, by Walker, Schmidt, Elsom & Johnson (1937) with creatinine on rabbits and dogs, and by Corcoran & Page (1939) with inulin and phenol red on dogs. Measurements of the renal blood flow by means of a thermostromuhr after the injection of posterior pituitary extract, or its vasopressor fraction, have been recorded by Janssen & Rein (1928), Geiling, Herrick & Essex (1934), Handovsky & Samaan (1937), Walker *et al.* (1937) and Wakim, Herrick, Baldes & Mann (1942). Recently developed clearance methods are likely to yield a more complete and more accurate picture of renal changes after an injection of posterior pituitary extract, since they permit the simultaneous measurement of glomerular filtration rate, effective renal blood flow and tubular activity in unanaesthetized and intact animals. A further clarification of the action of posterior pituitary extracts on the kidney, particularly with reference to the effect on the urinary excretion of chloride, can be expected from the results of clearance experiments made after the injection of the vasopressor and oxytocic fractions. Kuschinsky & Bundschuh's (1939) and Fraser's (1942) finding that the oxytocic fraction is more active than the vasopressor fraction in increasing the urinary chloride excretion will be remembered in this connexion. However, the mechanism of this effect was not investigated by these authors.

METHODS

Male adult albino rats were used throughout. The animals were kept on a standard diet (Vitamin A Test Diet, *U.S. Pharmacopoeia*, 11, revised 1937, with the addition of cod-liver oil and tocopherol) for some time before and during the period of experimentation.

All injections of posterior pituitary extract, or its fractions, were made subcutaneously and immediately after a dose of water, equal to 5% of the animal's body weight, had been given by

stomach tube. The posterior pituitary preparations were pituitrin, pitressin and pitocin, supplied by Messrs Parke, Davis and Co. The clearance estimations were performed when the rate of urine flow was sufficiently high to ensure short urine collecting periods (15–20 min.), i.e. about 45 min. after injection of pitocin, about 120 min. after injection of pitressin and about 60 min. after injection of pituitrin.

The experimental procedures for the determination of simultaneous inulin and diodone clearances in rats have been described in a previous paper (Dicker & Heller, 1945). Inulin in plasma and urine was determined by the method of Smith, Goldring & Chasis (1938). Diodone iodine in plasma and urine was determined by Alpert's (1941) method. Inulin (Kerfoot and Co.) and Per-Abrodil (Bayer Products Ltd.) were used. Chloride in plasma was estimated by Whitehorn's (1921) method, chloride in urine by that of Volhard-Arnold and expressed as NaCl. Definition and method of calculation of glomerular filtration rate (GFR = inulin clearance = C_{IN}), effective renal plasma flow (RPF), and total tubular excretory mass (Tm_D) conform to those outlined in a previous paper (Dicker & Heller, 1945). The fraction of plasma filtered through the glomeruli (filtration fraction = FF) was determined by dividing the filtration rate by the renal plasma flow. The rate of the tubular reabsorption of chloride (T_{Cl}) was calculated as follows:

$$T_{Cl} = (P_{Cl} \times C_{IN}) - (U_{Cl} \times V),$$

where P_{Cl} = concentration of plasma chloride in mg./100 ml., U_{Cl} = concentration of urinary chloride in mg./100 ml. and V = urine flow in ml./min. In order to permit the comparison of T_{Cl} values obtained at different values of C_{IN}, T_{Cl} was expressed as the percentage of chloride filtered.

Statistical treatment of results. Fisher's 't' test was applied to estimations of the significance of differences of means. 'Small sample' methods were used for the calculation of 't' and the correlation coefficient (r) for populations smaller than twenty (Mainland, 1938). Allowance for the number of samples in any one series of experiments was made by determining the probability (P) of 't' or 'r' from the tables of Fisher & Yates (1943).

RESULTS

Inulin, diodone and chloride clearances in normal rats. To provide a basis of comparison for the experiments with the posterior pituitary fractions, inulin, diodone and chloride clearances were estimated in a series of normal rats. The animals received 5% of their body weight of water by stomach tube. The following mean values and standard deviations were obtained (some previously published results (Dicker & Heller, 1945) of inulin and diodone clearance determinations were included): $GFR = 0{\cdot}347 \pm 0{\cdot}0432$ ml./100 g./min. (134 observations), $RPF = 2{\cdot}222 \pm 0{\cdot}2812$ ml./100 g./min. (28), $FF = 0{\cdot}17 \pm 0{\cdot}037$ (28), $Tm_D = 0{\cdot}1324 \pm 0{\cdot}01848$ mg. I/100 g./min. (84), $T_{Cl} = 97{\cdot}1 \pm 1{\cdot}51\%$ (41). There was no significant correlation between T_{Cl} and rate of urine flow ($r = +0{\cdot}012$, S.E. = $\pm 0{\cdot}156$, $P > 0{\cdot}1$), in other words the decrease in the concentration of urinary chloride which accompanied an increase of urine flow was not due to a change of the chloride reabsorption but solely due to the increase of urinary volume.

The plasma chloride concentration (P_{Cl}) varied little from animal to animal (mean $P_{Cl} = 356{\cdot}2$, S.E. = $\pm 18{\cdot}40$ mg./100 ml.).

The effect of injections of pitocin on inulin, diodone and chloride clearance. A series of twenty-one rats received 5% of their body weight of water by stomach tube and 3 mU./100 g. pitocin by subcutaneous injection. The

clearance estimations showed a significant increase of *GFR* and *RPF* (Fig. 1 A) but no change in the filtration fraction. The mean and standard deviation for *GFR* was $0 \cdot 624 \pm 0 \cdot 2373$ ml./100 g./min. A statistical comparison of this figure with the mean *GFR* of normal rats gave $t = 4 \cdot 461$, $P < 0 \cdot 001$, i.e. a highly significant difference. The mean and standard deviation for *RPF* was

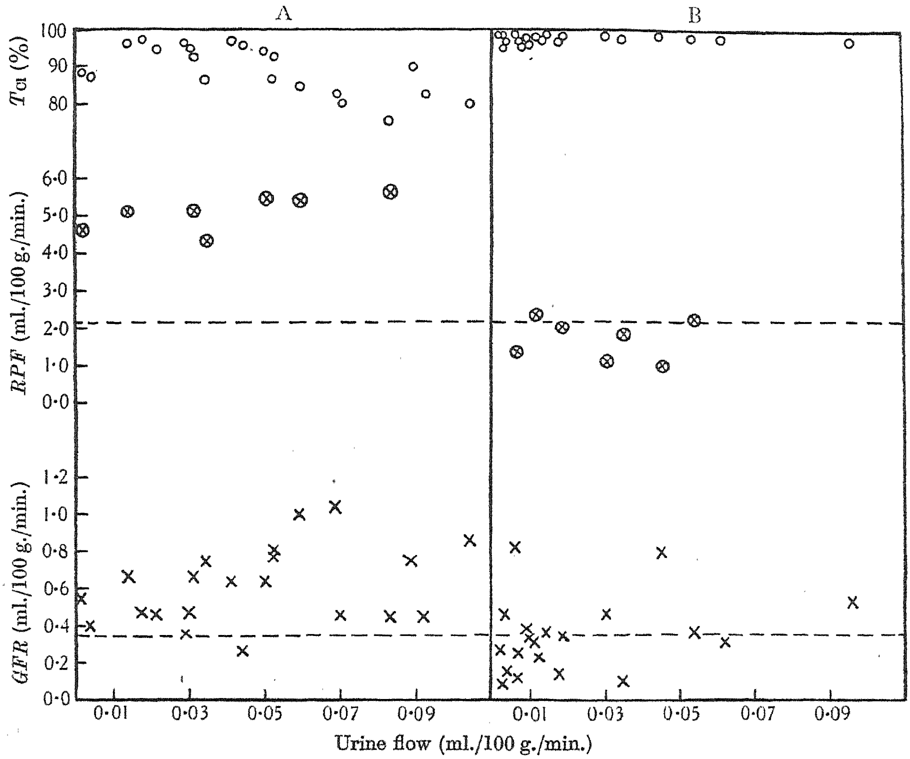

Fig. 1. A, renal effects of single subcutaneous injections of 3 mU./100 g. 'pitocin' on unanaesthetized rats hydrated with 5% of their body weight of water. B, renal effects of single subcutaneous injections of 3 mU./100 g. 'pitressin' on unanaesthetized rats hydrated with 5% of their body weight of water. × = glomerular filtration rate (*GFR*); ⊗ = effective renal plasma flow (*RPF*); ○ = rate of tubular chloride reabsorption as percentage of chloride filtered (T_{Cl}). The broken lines indicate the mean values obtained in non-injected controls.

$5 \cdot 230 \pm 0 \cdot 4679$ ml./100 g./min.; t between this figure and that for the mean *RPF* of the control animals was 16·683 ($P < 0 \cdot 001$). The mean value for *FF* was $0 \cdot 17 \pm 0 \cdot 053$. The figures for Tm_D (mean $Tm_D = 0 \cdot 1471$ mg. I/100 g./min., S.D. $= \pm 0 \cdot 02890$) were much the same as those obtained in the normal rats (t between the mean Tm_D of the 'pitocin animals' and that of the controls $= 1 \cdot 425$, $P > 0 \cdot 1$).

The results of the determinations of the rate of tubular chloride reabsorption (T_{Cl}) gave a mean of $91 \cdot 5 \pm 2 \cdot 15\%$, a figure which differed very significantly

from that obtained in the normal series ($t = 10 \cdot 470$, $P < 0 \cdot 001$). The rate of tubular chloride reabsorption in the pitocin animals was significantly correlated to the rate of urine flow ($r = -0 \cdot 581$, s.e. $= \pm 0 \cdot 235$, $P < 0 \cdot 01$). The mean plasma chloride concentration in the experiments with pitocin was $379 \cdot 2 \pm 10 \cdot 09$ mg./100 ml.; it did not differ significantly from that of the normal rats ($t = 1 \cdot 197$, $P > 0 \cdot 2$). There is, therefore, no evidence to ascribe the decreased T_{Cl} after pitocin to anything but a renal effect.

The interpretation of the results obtained with the dose of pitocin used (3·0 mU./100 g. rat) is complicated by the fact that this preparation contains appreciable amounts (5–10%) of the vasopressor-antidiuretic factor (Kamm, Aldrich, Grote, Rowe & Bugbee, 1928; Moir, 1944). Control experiments with 0·3 mU./100 g. pitressin were therefore performed.

The effect of injections of pitressin on inulin, diodone and chloride clearance. Twelve rats received 5% of their body weight of water by stomach tube and 0·3 mU./100 g. pitressin by subcutaneous injection. Inulin and diodone clearance estimations in these animals gave the following results (means and s.d.): $GFR = 0 \cdot 735 \pm 0 \cdot 2226$ ml./100 g./min., $RPF = 4 \cdot 670 \pm 0 \cdot 9141$ ml./100 g./min., $FF = 0 \cdot 20 \pm 0 \cdot 036$. The means for GFR and RPF are significantly different from the respective mean values obtained in the control animals (t for $GFR = 6 \cdot 933$, $P < 0 \cdot 001$; t for $RPF = 8 \cdot 023$, $P < 0 \cdot 001$), showing that a dose of pitressin as small as 0·3 mU./100 g. rat had a pronounced renal effect.

A comparison of the results obtained with 0·3 mU./100 g. pitressin with those obtained with 3·0 mU. pitocin showed that there was no significant difference between the mean values for GFR ($t = 1 \cdot 441$, $P > 0 \cdot 1$) and RPF ($t = 1 \cdot 488$, $P > 0 \cdot 1$) [the values for FF of the two series were also of similar magnitude $t = 1 \cdot 282$, $P > 0 \cdot 2$], i.e. injections of 0·3 mU./100 g. pitressin and 3·0 mU./100 g. pitocin had much the same effect on glomerular filtration rate and renal plasma flow. It would appear from this that an admixture of about 10% of the vasopressor-antidiuretic factor to the oxytocic principle could account for the increase of GFR and RPF observed after the injection of 'pitocin'.

The renal effect of injection of 0·3 mU. pitressin differed from that following injection of 3·0 mU. pitocin in that pitressin failed to have an action on the rate of chloride reabsorption by the tubules (t between T_{Cl} of the 0·3 mU. pitressin series and that of the control series $= 0 \cdot 300$, $P > 0 \cdot 8$). It could further be shown that this absence of a significant effect on the rate of tubular chloride reabsorption applied also when much higher doses of pitressin were injected (Fig. 1 B). The mean value for the rate of chloride reabsorption in twenty rats injected with 3·0 mU./100 g. pitressin was $98 \cdot 3 \pm 1 \cdot 35$%. This result was not significantly different from that obtained in the control series ($t = 1 \cdot 650$, $P > 0 \cdot 2$), showing again that the vasopressor-antidiuretic fraction had no effect on the rate of tubular chloride reabsorption.

Determinations of inulin and diodone clearances in the rats injected with 3·0 mU./100 g. pitressin gave the following results (means and S.D.): $GFR = 0{\cdot}352 \pm 0{\cdot}2010$ ml./100 g./min., $RPF = 1{\cdot}775 \pm 0{\cdot}5330$ ml./100 g./min., $FF = 0{\cdot}32 \pm 0{\cdot}249$, $Tm_D = 0{\cdot}0973 \pm 0{\cdot}03793$ mg. I/100 g./min. Considering the pronounced increase of *GFR* after the injection of 0·3 mU./100 g. pitressin it seemed surprising, at first, that the mean value for *GFR* of animals which had received a dose ten times higher was much the same as that of normal controls. However, a comparison of the coefficient of variation of *GFR* of the 3·0 mU./100 g. pitressin series (=57·1 %, S.E. = ±9·03) with that of the control series (=12·4 %, S.E. = ±0·76) showed a highly significant difference (44·7 %, S.E. = ±9·06), implying that 3·0 mU./100 g. pitressin did have an effect on the rate of glomerular filtration. Furthermore, the difference between the coefficient of variation of *RPF* in this series (=30·0 %, S.E. = ±8·05) and that of the control animals (=2·6 %, S.E. = ±0·04) was also significant (difference between coefficients of variation = 27·4 ± 8·06 %). From this figure it would seem likely that the increased variability of *GFR* after the injection of 3·0 mU./100 g. pitressin was, partly at least, due to a vascular effect.

The effect of injections of pituitrin on inulin, diodone and chloride clearance. Next it seemed of interest to compare these effects of the posterior pituitary fractions with the effects of the undifferentiated posterior pituitary extract. The standard mammalian extract contains the oxytocic and vasopressor-antidiuretic factors in equal proportions. The dose of 3·0 mU./100 g. 'pituitrin' was, therefore, chosen. The following results were obtained in a series of forty clearance experiments (means and S.D.): $GFR = 0{\cdot}379 \pm 0{\cdot}1460$ ml./100 g./min. (coefficient of variation = 38·5 %, S.E. = ±4·30), $RPF = 1{\cdot}705 \pm 0{\cdot}6427$ ml./100 g./min. (coefficients of variation = 31·8 %, S.E. = ±4·8), $FF = 0{\cdot}23 \pm 0{\cdot}131$, $Tm_D = 0{\cdot}1047 \pm 0{\cdot}04278$ mg. I/100 g./min.

The difference between the coefficient of variation of the mean *GFR* of this series and that of the animals injected with 3·0 mU./100 g. pitressin was not significant (18·6 %, S.E. = ±9·99), nor was there any significant difference between the coefficients of the mean *RPF* of the two series (1·8 %, S.E. = ±9·37). These results suggest that the changes of variability of *GFR* and *RPF* after the injection of 3·0 mU./100 g. pituitrin were essentially due to the pitressin content of this preparation.

The mean value of T_{Cl} for the pituitrin series was 94·9 %, S.D. = ±4·44. This figure differs significantly from that obtained in the controls ($t = 2{\cdot}983$, $P < 0{\cdot}01$). Since it has been shown that pitocin lowers the rate of tubular chloride reabsorption whereas pitressin does not influence it significantly, it seems justifiable to assume that the effect of pituitrin on the tubular chloride reabsorption was due to the oxytocic principle contained in the undifferentiated posterior pituitary extract.

DISCUSSION

A substantial separation of the posterior pituitary principles has been effected in the preparations 'pitocin' and 'pitressin', but it should be noted that the differentiation is not complete and that each of these extracts contains 5–10 % of the other, reckoned in units of respective activity. 'Pitocin' and 'pitressin' are therefore not synonymous with the pure oxytocic or vasopressor-antidiuretic principles.

A comparison of the effects of small subcutaneous doses of pitocin and pitressin (Table 1) shows that pitocin decreased the rate of tubular reabsorption

TABLE 1. Renal effects of injections of posterior pituitary extract and its fractions. The figures are mean results with their standard deviations

	GFR (inulin clearance) ml./100 g./min.	*RPF* (diodone clearance) ml./100 g./min.	*FF* (*GFR*/*RPF*)	Tm_D mg. I/100 g./min.	T_{Cl} (as % of chloride filtered)
Controls	0·347±0·0432	2·222±0·2812	0·17±0·037	0·1324±0·01848	97·1±1·51
3·0 mU./100 g. pitocin	0·624±0·2373	5·230±0·4679	0·17±0·053	0·1471±0·02890	91·5±2·15
0·3 mU./100 g. pitressin	0·735±0·2226	4·670±0·9141	0·20±0·036	—	98·6±0·73
3·0 mU./100 g. pitressin	0·352±0·2010	1·775±0·5330	0·32±0·249	0·0973±0·03793	98·3±1·35
3·0 mU./100 g. pituitrin	0·379±0·1460	1·705±0·6427	0·23±0·131	0·1047±0·04278	94·9±4·44

of chloride (T_{Cl}) by the rat kidney while pitressin had no effect on this process. It may, therefore, be concluded that the depression of the rate of tubular chloride reabsorption was due to the posterior pituitary oxytocic principle.

However, it is more difficult to decide whether the increase of glomerular filtration rate (*GFR*) and of effective renal plasma flow (*RPF*), found after the injection of 3·0 mU./100 g. pitocin, was due to the oxytocic factor or to the residual vasopressor-antidiuretic activity contained in pitocin. Table 1 shows that injections of a very small dose of pitressin (0·3 mU./100 g. rat) produced rises of *GFR* and *RPF* which were statistically comparable with those produced by 3·0 mU./100 g. pitocin. It would seem, therefore, that the oxytocic factor, in the doses given, did not affect *GFR* and *RPF* (compare also the results of injections of 3·0 mU./100 g. pituitrin with those of 3·0 mU./100 g. pitressin). Had the effect on *GFR* and *RPF* of injections of 3·0 mU. pitocin been due to the oxytocic principle, why did it occur after the injection of 0·3 mU. pitressin which contains presumably not more than 0·03 mU. of the oxytocic factor, and why did it not occur after the injection of 3·0 mU. pituitrin which contains 3·0 mU. of the oxytocic substance?

It may be concluded from this comparison that any effects on *RPF* and *GFR* were due to the vasopressor principle, but it will be seen that these

effects varied with the amount of the vasopressor substance injected. Injections of the lower dose (0·3 mU./100 g.) of pitressin produced an increase of *RPF* and *GFR* (Table 1) explainable, for instance, by a dilator action on the glomerular arterioles. The response to the higher dose (3·0 mU./100 g.) lacked uniformity: the values for *GFR* and *RPF* in individual animals were frequently either significantly lower or significantly higher than those of normal rats. It is understandable that such differences from the normal did not find an expression in the mean values for *GFR* and *RPF* of the series. However, they appear in a highly significant difference between the coefficients of variation of the means. There is a similarity between these results on rats and those of Wakim *et al.* (1942), who measured the renal blood flow in dogs after the injection of pitressin. Their measurements, which were made with a thermostromuhr, showed decreases of the renal blood flow after intravenous injections, but increases were observed when pitressin was administered intramuscularly. These findings agree with the results of the present clearance experiments on rats in suggesting that the renal vascular effect of pitressin may vary in character according to the pitressin concentration in the blood.

SUMMARY

1. Glomerular filtration rate (*GFR*), effective renal plasma flow (*RPF*) and rate of tubular chloride reabsorption were measured by simultaneous inulin, diodone and chloride clearances in unanaesthetized rats.

2. Subcutaneous injections of 3 mU./100 g. pitocin produced a significant depression of the rate of tubular chloride reabsorption and a significant increase of *GFR* and *RPF*.

3. Subcutaneous injections of 3 mU./100 g. pitressin had no effect on the rate of tubular chloride reabsorption. However, they produced a significantly increased variability of *GFR* and *RPF*.

4. Subcutaneous injections of 3 mU./100 g. of undifferentiated posterior pituitary extract (pituitrin) produced changes of *GFR* and *RPF* of the same magnitude as those observed after 3 mU./100 g. pitressin, and a significant decrease of the rate of chloride reabsorption similar to that observed after the injections of pitocin.

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FACTORS INFLUENCING DEPOSITION OF GLYCOGEN IN ADIPOSE TISSUE OF THE RAT

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Injection of insulin induces deposition of fat in the adipose tissue of normal rats. This action is preceded by the appearance of glycogen within the fatty tissue (Wertheimer, 1945). Wasting of adipose tissue is a conspicuous feature of severe pancreatic diabetes in man. Stetten & Boxer (1944) found that rats, after injection of alloxan, utilize glucose for fatty acid synthesis at a rate which is only 5% of the normal. Pauls & Drury (1942) believe that depancreatized rats, after insulin administration, store most of the ingested carbohydrate as fat. Experiments by the writers (Tuerkischer & Wertheimer, 1942; Wertheimer, 1945) showed that transformation of carbohydrate into fat is always preceded by deposition of glycogen in adipose tissue. It seemed therefore to be of interest to find whether glycogen also appears in the adipose tissue of rats made diabetic with alloxan and, if not, whether insulin administration would restore the ability of the diabetic organism to deposit glycogen in the adipose tissue.

The following types of experiments were carried out:

I. Glycogen was estimated in the adipose tissue (*a*) of diabetic rats, and (*b*) of diabetic rats kept on a carbohydrate-rich diet and treated with insulin.

II. Glycogen was estimated in the adipose tissue of diabetic rats maintained under conditions which lead, in normal rats, to an intensive synthesis of fat from carbohydrate with a prior deposition of glycogen in the adipose tissue. Such conditions are: (*a*) Prolonged starvation followed by a diet rich in carbohydrate (Tuerkischer & Wertheimer, 1942). (*b*) Controlled feeding regimens (i) of MacKay & Drury (1941) and of Wertheimer (1945); (ii) of Teppermann, Brobeck & Long (1943) known for convenience as 'Long' experiments.

Teppermann *et al.* (1943) found that rats trained to eat an entire daily ration in 1–2 hr. had a mean R.Q. of 1·22 after administration of carbohydrate food. Animals allowed access to the same amount of food throughout day and night had an R.Q. of about 1·05. Werthessen (1937) found that trained

rats can have an R.Q. as high as 1·7 in the period of absorption. These experiments were thought to show that dietary training can augment the rate of fatty acid synthesis from carbohydrate.

METHODS

Animals. Young male albino rats of laboratory stock were used. The diabetic rats weighed 90–110 g., the adrenalectomized rats weighed 110–150 g.

Diets. The standard diet consisted of wheat and vegetables. The carbohydrate-rich diet had 70% starch, 20% casein, 10% fat with the usual supplements of 'minerals' and vitamins. In forced feeding experiments solutions were given by stomach tube. Such solutions had merely 25% glucose or glucose 42 g., sucrose 42 g., dried egg 15 g., carbo animalis 5 g., Osborne-Mendel salt mixture 4 g., water to 150 c.c. (Samuels, Reinecke & Ball, 1942).

Procedure. Diabetes was produced by injection of alloxan. Fat digestion was unaffected. Gomori & Goldner (1943) and Duff & Starr (1944) injected doses of 200 mg./kg. body weight intraperitoneally. We injected 60–80 mg. alloxan/kg. body weight intravenously. Diabetes was induced in 145 out of 151 rats. More or less pronounced acetonuria occurred in thirteen of the rats. Polyuria and glycosuria often appeared together. Larger doses of alloxan did not lead to aggravation of the diabetic signs. Smaller doses (40–50 mg. alloxan/kg. body weight) were not consistently effective. The evidence that alloxan diabetes is a true pancreatic diabetes is fairly conclusive. The following feature, however, is worthy of emphasis. When the rats were starved, we often observed a return of the blood-sugar level to normal with disappearance of glycosuria. The diabetes, however, was still present in a latent form. Burn, Lewis & Kelsey (1944) have recently described much the same phenomenon, the disappearance of glycosuria in alloxan-diabetic rats after administration of food rich in fat. Hyperglycaemia can be re-established in such rats by administration of food rich in carbohydrate. A rise of the blood-sugar level to diabetic values, with glycosuria, can be brought about in such rats, even if starvation be continued, by administration of adrenal cortex extract. In our experiments we injected 1·5 mg./100 g. body weight of Kendall's Compound B.

Experiments in which a diet rich in carbohydrate was given after prolonged starvation were carried out as described previously by Tuerkischer & Wertheimer (1942). Experiments according to MacKay & Drury (1941) were carried out as described by Wertheimer (1945). In 'Long' experiments, rats were trained for 10–14 days to eat their full daily ration during 2 hr. Only animals which maintained their body weight during training were used for experiment. Alloxan was injected at the end of the preparatory period. Food was withheld after the injection until the following day. In these experiments, in order to ensure equal food intake by both the control diabetic and the insulin-treated rats, the final carbohydrate-rich diet was given by forced feeding. In experiments after prolonged starvation, and in experiments according to MacKay & Drury (1941), forced feeding was also used. Simultaneously with the forced feeding certain rats received an injection of 4 units of Protamine-Zinc-Insulin.

Protamine-Zinc-Insulin was employed for most of the experiments. Ordinary insulin was used for some experiments of short duration.

The methods used to prepare the fat samples, and the chemical procedures employed, are described in our former papers (Tuerkischer & Wertheimer, 1942; Wertheimer, 1945).

RESULTS

Effect of insulin in alloxan-diabetic rats

In normal rats, excessive ingestion of carbohydrate foods, prolonged starvation, involving losses up to 20% of the body weight, with subsequent carbohydrate-rich feeding, and controlled feeding according to MacKay & Drury, all induce

TABLE 1. Glycogen deposition in adipose tissues of alloxan-diabetic rats

Treatment	Animals	No. of exps.	Duration of exp. hr.	Glycogen in g./100 g. in Liver	Muscle	'Mixed' fat	Mesentery fat	Interscapular fat	Blood sugar mg./100 c.c.
Excess carbohydrate intake	Controls	19	$\frac{3}{4}$–10	0·99 ± 0·21	0·20 ± 0·02	0	0	0	619 ± 50
	Insulin	20	$\frac{3}{4}$–10	1·80 ± 0·20	0·42 ± 0·003	Traces	Traces	1·08 ± 0·14	247 ± 50
	Controls	4	17–23	2·79 ± 1·10	0·35 ± 0·07	0	0	0	525 ± 127
	Insulin	4	17–23	4·72 ± 0·41	0·55 ± 0·05	0·11 ± 0·05	0·13 ± 0·02	1·88 ± 0·14	86 ± 28
Feeding after starvation	Controls	5	5– 7	1·08 ± 0·49	0·24 ± 0·04	0	0	0	803 ± 49
	Insulin	4	5– 7	2·40 ± 0·59	0·71 ± 0·12	Traces	0·07 ± 0·12	1·93 ± 0·31	332 ± 74
	Controls	13	22–54	2·03 ± 0·31	0·30 ± 0·04	0	0	Traces	735 ± 96
	Insulin	14	22–54	4·05 ± 0·30	0·63 ± 0·03	0·11 ± 0·04	0·24 ± 0·07	1·08 ± 0·26	142 ± 51
Exps. according to MacKay & Drury	Controls	6	6– 7	2·38 ± 0·47	0·28 ± 0·05	0	0	0	576 ± 92
	Insulin	6	6– 7	2·90 ± 0·47	0·60 ± 0·04	0·06 ± 0·01	0·09 ± 0·04	0·78 ± 0·04	59 ± 9
Exps. according to Long	Controls	8	6– 7	2·40 ± 0·66	0·29 ± 0·02	0	0	0·07 ± 0·04	651 ± 104
	Insulin	6	6– 7	2·89 ± 0·46	0·48 ± 0·001	Traces	0·18 ± 0·05	1·06 ± 0·33	57 ± 4

deposition of glycogen in the adipose tissue prior to the formation of fat in the same tissue. No such deposition took place in rats made diabetic with alloxan. However, deposition of glycogen in the interscapular adipose tissue was induced in alloxan-diabetic rats by insulin administration even during starvation lasting 2–10 hr. Glycogen was found in thirteen of twenty animals treated, the values ranging from 0·025 to 1·13 g./100 g. fat tissue.

In controlled feeding experiments according to Long, a group of sixty normal rats showed the following results: Glycogen appeared within the interscapular adipose tissue 1 hr. after, and in the mesentery and 'mixed' fat 2–3 hr. after, food was taken. This glycogen deposition reached its peak level after 7–8 hr., the glycogen values then being 2·8 g./100 g. in the interscapular fat, 0·5 g./100 g. in the mesentery fat and 0·16 g./100 g. in the 'mixed' fat. Sixteen to eighteen hours later no glycogen could be detected in the adipose tissues. The results of similar experiments with alloxan-diabetic and with insulin-treated alloxan-diabetic rats are given in Table 1. Table 2 summarizes these findings.

TABLE 2. Glycogen deposition in adipose tissue

Treatment	Normal rats	Alloxan-diabetic rats	Alloxan-diabetic insulin-treated rats
Starvation	–	–	+
Carbohydrate-rich diet	+ (only when given in excess)	–	+ + +
Feeding following starvation	+ +	–	+ + +
Exps. according to MacKay & Drury	+ +	–	+ +
Exps. according to Long	+ +	–	+ +

Effect of insulin in adrenalectomized rats

As shown in previous experiments (Tuerkischer & Wertheimer, 1942) adrenalectomized rats are unable to deposit either glycogen or fat in the adipose tissue when given carbohydrate-rich food after prolonged starvation.

When adrenalectomized rats are kept on a carbohydrate-rich diet and receive 25% glucose solution rather than water to drink, glycogen deposition can be induced in all adipose tissues by insulin administration even without previous starvation. When insulin treatment is continued this ability disappears after about 3 days. In contrast to the finding in normal rats, the appetite for carbohydrate on the part of the adrenalectomized animals was not increased by insulin administration. The results of these experiments are given in Table 3.

TABLE 3. Glycogen deposition in the adipose tissues of adrenalectomized rats after insulin administration

					Glycogen in g./100 g. in					
nimals	No. of exps.	Insulin dose; units twice daily	Duration of exp. days	25% glucose soln. drunk c.c.	Liver	Muscle	'Mixed' fat	Mesentery fat	Interscapular fat	Blood sugar mg./100 c.c.
ontrols	7	—	1	27	0·87 ± 0·27	—	0	0	0	104 ± 8
nsulin	10	1–3	1	26	1·48 ± 0·22	—	0·26 ± 0·09	0·46 ± 0·17	0·16 ± 0·04	63 ± 5
ontrols	4	—	2½–3	42	0·97 ± 0·11	0·30 ± 0·01	0	0	0	98 ± 11
nsulin	5	0·5–1	2½–3	41	1·54 ± 0·31	0·38 ± 0·04	0·09 ± 0·03	0·30 ± 0·07	0·20 ± 0·04	51 ± 9

Influence of adrenaline given together with insulin

Two injections of 0·02–0·03 mg. adrenaline per 100 g. rat were given simultaneously with insulin in an experimental period of 4–8 hr. Inhibition of glycogen deposition in the adipose tissue was noted in a total of eight experiments with both normal and diabetic rats. Smaller doses of adrenaline, 0·005–0·015 mg./100 g. rat, caused a reduction in the amount of glycogen deposition induced by insulin.

Influence of a dead and washed preparation of Salmonella typhi murium *('endotoxin') given together with insulin*

'Endotoxin' of *Salmonella typhi murium* suppresses the deposition of glycogen in the adipose tissue following excess carbohydrate feeding after prolonged starvation (Tuerkischer & Wertheimer, 1942). Administration of 1 mg. of 'endotoxin' either entirely suppressed or decreased the deposition of glycogen in adipose tissue after injection of insulin. The inhibition lasted for 7–14 hr. and was observed in seven experiments.

DISCUSSION

In alloxan-diabetic rats the glycogen reserve of the muscle and of the liver was approximately doubled by insulin administration. In controlled feeding experiments, however, the liver glycogen content of the diabetic rats was already relatively high and could be increased only to a small degree by insulin administration. Complete inability to synthesize glycogen, therefore, seems to be limited in alloxan diabetes to the adipose tissue. Our experiments, in conjunction with those of Stetten & Boxer (1944) and of Pauls & Drury (1942), demonstrate the connexion between deposition of glycogen in adipose

tissue and synthesis of fat from carbohydrate. It must be concluded that alloxan diabetes disturbs the metabolism of adipose tissues and that this disturbance can be corrected by insulin.

The experiments of Dickerson, Teppermann & Long (1943) are consistent with the hypothesis, which is generally accepted but which has never been proved, that synthesis of fatty acids from carbohydrate takes place in the liver. On the other hand, Teppermann *et al.* (1943) have shown, in experiments with practically liverless rats, that extrahepatic factors participate in synthesis of fat from carbohydrate. The demonstrated connexion between glycogen deposition in adipose tissues and the synthesis of fat from carbohydrate—a connexion now shown to hold also in alloxan-diabetic rats—indicates that adipose tissue plays a part in the synthesis.

SUMMARY

1. Alloxan-diabetic rats are unable to synthesize glycogen in the adipose tissues under conditions which induce deposition of glycogen in the adipose tissues of normal rats. Administration of insulin fully restores the ability of alloxan-diabetic rats to deposit glycogen in their adipose tissues.

2. In adrenalectomized rats insulin administration induces deposition of glycogen in the adipose tissue.

3. Glycogen deposition in adipose tissue under the influence of insulin is prevented by simultaneous injection of adrenaline.

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TEMPERATURE AND BLOOD FLOW IN THE HUMAN FOREARM

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The temperature conditions and the rate of blood flow in the human forearm can be affected by alteration of the external temperature, in particular when the forearm is immersed in water at different temperatures (Barcroft & Edholm, 1943). In order to assess changes, it is useful to have some standard for comparison. The standard which has been selected is provided by the conditions existing in the adequately clothed forearm at rest, indoors, at a room temperature of 18–20° C. There have been many previous investigations of both temperature and blood flow in the forearm, but they have dealt mainly with the temperature of the forearm skin (Benedict, 1925; Bazett & McGlone, 1927; Lewis, 1927; Collier & Maddick, 1932), and though blood flow has been measured under various conditions, the limb has always been immersed in water. Bath temperatures of 25° C. (Prinzmetal & Wilson, 1936), 30° C. (Grant & Pearson, 1938), 32° C. (Abramson & Fierst, 1942) and 35° C. (Barcroft & Edholm, 1943) have been used.

The data presented in this paper enable a more rational choice of water-bath temperature to be made by comparing the temperature of the skin, subcutaneous tissue and muscle, and the blood flow of the whole forearm, in the clothed forearm and when the arm is immersed in water at different temperatures.

The opportunity has also been taken to measure the forearm temperature while the uncovered limb was (i) cooling in air and (ii) immersed in water at different temperatures.

METHODS

The subjects were healthy adult males, aged from 20 to 40 years. All experiments were done in a basement room at a mean temperature of 18·5° C., and where the humidity and movement of the air only varied slightly from day to day.

The subject wore his usual clothes in which he felt comfortably warm. The measurement of the temperature of the clothed forearm was made with the subject reclining in an armchair, his clothed forearm resting on the arm of the chair. Before the experiment the position of the veins on the forearm was marked out, since Lewis (1927) has shown that the temperature of the skin in the neighbourhood of a vein may be influenced by the temperature of the venous blood. After

15–20 min. the arm was bared, and the following procedures were carried out as quickly as possible. A hypodermic needle thermojunction 2·5 cm. long was thrust up to the hilt obliquely under the skin covering the brachioradialis muscle, with the tip in the subcutaneous tissue some distance from the nearest vein; a second needle (also 2·5 cm. long) was inserted vertically through the skin into the brachioradialis muscle with the tip near the radius, about 5 cm. distal to the head of the bone. This needle was pushed through the muscle down to the bone and then withdrawn about 1 mm., so that in all subjects temperature readings were taken in the deepest portion of the brachioradialis muscle. Skin temperature was measured thermoelectrically (Lewis, 1927) near the tip of the subcutaneous needle, but avoiding the vicinity of a vein or of an erythematous area apt to appear over the tip of the needle.

In some experiments the temperatures were measured at 5 min. intervals for 2 hr. while the forearm was exposed to the air. In others, subcutaneous and deep-muscle temperatures were taken while the limb was immersed in water. The procedure in these last experiments was the same as described by Barcroft & Edholm (1943).

When the blood flow was measured, cotton-wool was used to simulate clothing. A plethysmograph was fitted to the left forearm (Barcroft & Edholm, 1943). After the diaphragms had been cemented to the skin, the portion of the forearm between them was covered with cotton-wool, as was any exposed part of the arm above the upper diaphragm. The forearm, including the part in the plethysmograph, was in effect clothed. The routine arrangements for recording the blood flow were then completed except that the plethysmograph was not filled with water, and the limb was not immersed in the water-bath. The plethysmograph was carefully protected from draughts.

To ascertain whether the cotton-wool protected the limb from heat loss, a sleeve of it was fitted to the opposite arm, after a thermojunction needle had been inserted into the brachioradialis muscle. A small area of skin could be exposed for the measurement of skin temperature.

In some experiments a larger, 15 cm. diameter, plethysmograph was used. This enabled blood flow and temperature measurements to be made simultaneously on the same forearm. The lead for the thermojunction was taken through the upper rubber diaphragm and the needle was inserted into the limb inside the plethysmograph. The forearm was then covered with cotton-wool as above and the arrangements for recording completed. The thermojunction for the measurement of skin temperature could be manipulated from the outside of the plethysmograph on to a small bare area of skin within.

Blood flow and temperature were recorded every 5 min. for 2 hr.

RESULTS

Temperature measurements

Temperatures in the forearm immediately after baring. Readings of all three of the arm temperatures were obtained within 15–60 sec. of baring the arm, skin temperature being measured first (Table 1).

TABLE 1

	Average temp. °C.	No. of subjects
Skin	33·0 ±0·62	15
Subcutaneous tissue	33·6 ±0·91	26
Deep muscle	36·2 ±0·56	30
Mouth	36·86±0·11	30

In the fifteen subjects in whom all the three arm temperatures were measured, the averages for the subcutaneous and deep muscle temperature were identical with the larger series shown in Table 1.

The forearm exposed to air. Five subjects were investigated. The results are shown in Fig. 1. The rate of cooling of the deep-muscle layers varied considerably; in the 2 hr. of the experiment in one subject, the temperature here fell 7·4° C., in another, under identical conditions, the temperature only fell 3·7° C. On the other hand, subcutaneous and skin temperatures fell at similar rates in all subjects, the final values being 28·5 ± 0·60 and 27·9 ± 0·46° C.

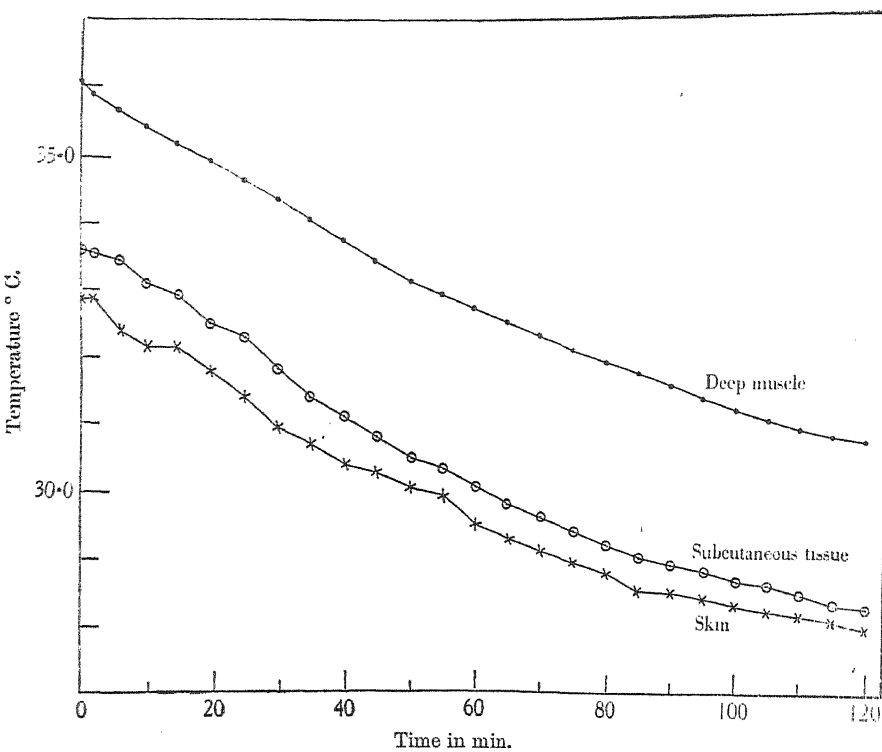

Fig. 1. The skin, subcutaneous and deep-muscle temperatures in the forearm exposed to room air at time 0.

The forearm immersed in water. The number of subjects investigated at each temperature is indicated in Fig. 2 together with the results. With the lower bath temperature there was little difference at the end of the 2 hr. period between the temperature of the bath and that of the subcutaneous tissue. With bath temperatures above body temperature, there was sometimes considerable difference between the subcutaneous temperature and that of the bath.

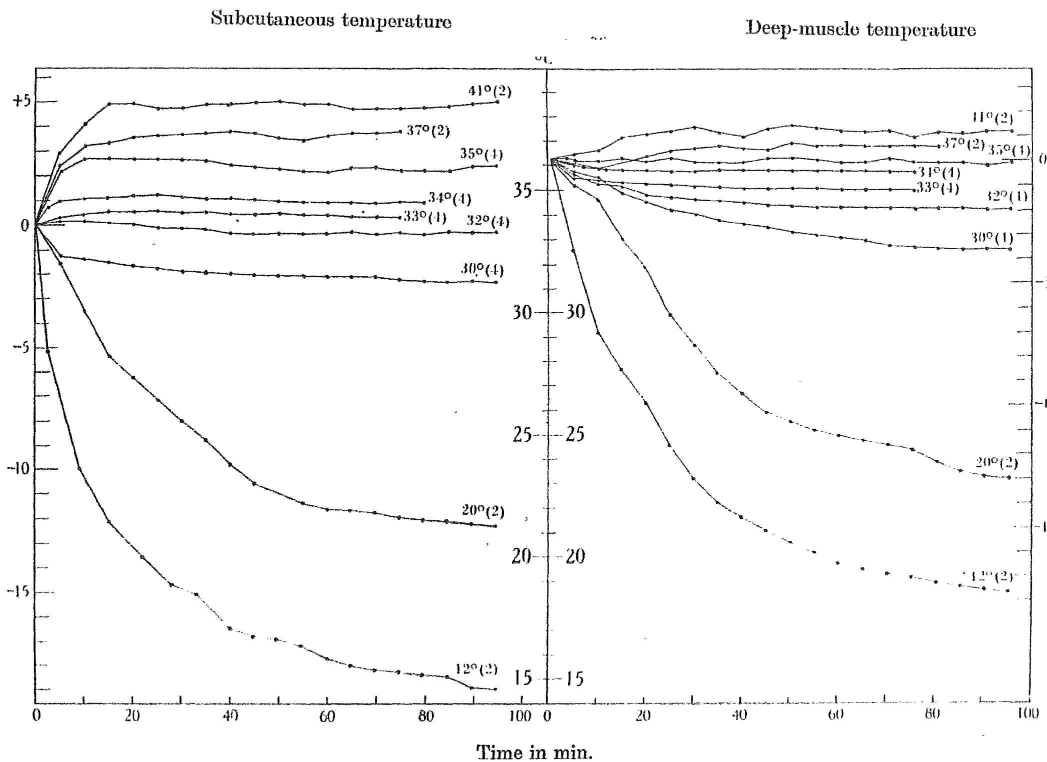

Fig. 2. The subcutaneous and deep-muscle temperatures in the forearm placed in the water-bath at time 0. The water-bath temperature is given above the right-hand end of each curve. In the centre of the figure the ordinates show the subcutaneous and deep-muscle temperature in ° C. On the right and left of the figure the ordinates are given as deviations, in ° C., from the average temperature in the clothed arm; on the left side 0 = 33·6° C., the average subcutaneous temperature in the clothed forearm; on the right 0 = 36·2° C., the average deep-muscle temperature.

Blood-flow measurements

Blood flow in the covered forearm. Seven subjects were investigated. The actual recording of the blood flow with the air-filled plethysmograph presented no difficulties. Fig. 3 shows a typical tracing. The results have been averaged

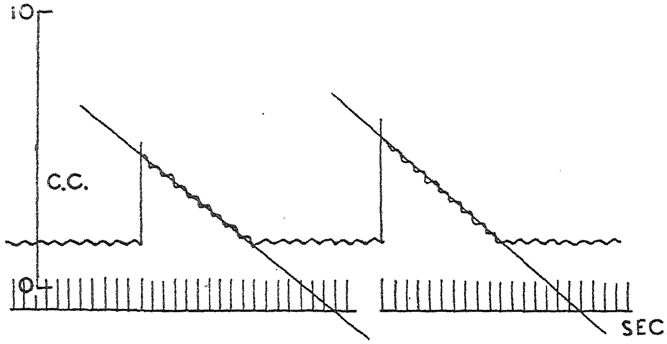

Fig. 3. Tracings of the blood flow in the clothed forearm recorded with the plethysmograph filled with air instead of water.

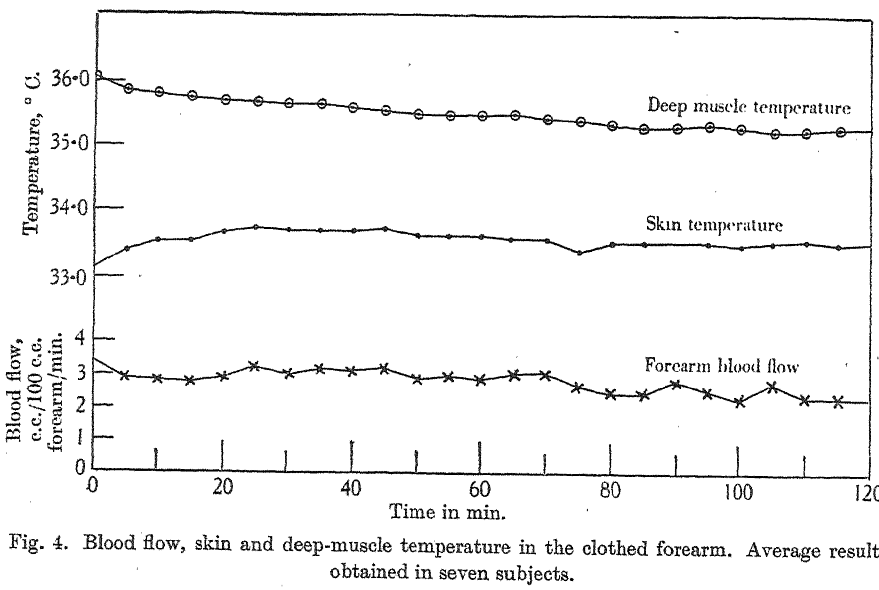

Fig. 4. Blood flow, skin and deep-muscle temperature in the clothed forearm. Average results obtained in seven subjects.

and are shown in Fig. 4. There was a slight rise in skin temperature and a slight fall in deep-muscle temperature over the 2 hr. period of the experiment. The cotton-wool sleeve provided adequate protection from heat loss, since forearm temperatures were maintained at a nearly steady level for 2 hr., and these temperatures were similar to those found in the forearm immediately after baring. During the first 50 min. the blood flow was fairly constant at

$3 \cdot 1 \pm 0 \cdot 25$ c.c./100 c.c. forearm/min. As will be seen from the small standard deviation there was little difference between the seven subjects. During the last 50 min. of the experiment the average figure was only 2·6 c.c. The fall in the rate of blood flow can be accounted for partly by the slight decline in muscle temperature. The figure 3·1 c.c. has been taken as the average blood flow in the clothed arm and is that shown in Table 2.

TABLE 2

	Average blood flow c.c./100 c.c. forearm/min.	Temperature ° C.		
		Skin	Subcutaneous	Deep muscle
Forearm clothed	3·1	33·0	33·6	36·2
Forearm in the water-bath at: 37° C.	5·9	37·0	37·2	36·8
35° C.	4·25	35·0	36·0	36·2
34° C.	3·4	34·0	34·7	35·8
33° C.	2·7	33·0	34·0	35·0
32° C.	2·3	32·0	33·3	34·3
30° C.	1·6	30·0	31·4	32·8

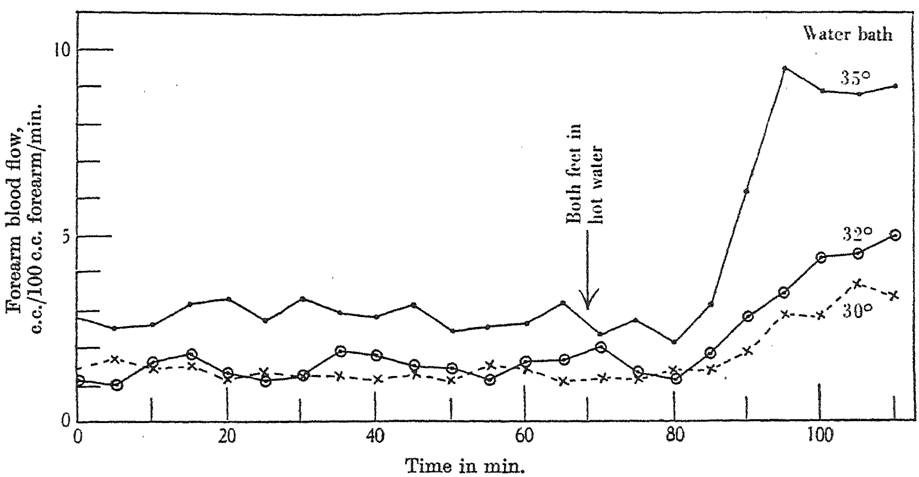

Fig. 5. The effect on forearm blood flow of placing the feet in water at 44–45° C., measured on three separate occasions in the same subject. The forearm was in the water-bath at the water temperature indicated. An increased flow is demonstrated in every case on heating the feet and the effect is considerably emphasized when the forearm bath temperature is raised.

Similar results were obtained when the blood flow and temperature measurements were made on the same arm.

Effect of water-bath temperature on response of forearm blood vessels. In one subject the effect of heat applied to the lower limbs on forearm blood flow was measured. On different occasions the test arm was kept in a water-bath at 30·0, 32·0 and 35·0° C. After a control period of 30 min., the feet and ankles were immersed in water kept between 44·0 and 45·0° C. This produced a rise

in body temperature and an increased blood flow in the forearm. The results are shown in Fig. 5. It will be seen that an increased flow was obtained at all three water-bath temperatures, but that the increase was only slight when the arm was in water at 30·0° C., whereas it became progressively greater as the water-bath temperature was raised. The rise in body temperature provoked by the heating of the feet was identical in all three cases.

DISCUSSION

The average temperature of the forearm skin immediately after baring is 33·0° C. This figure is slightly higher than that of 32·4° C. obtained by Benedict (1925). Collier & Maddick (1932) found the skin temperature of the forearm to average 34·0° C. in subjects heavily clothed in sheet rubber. In an environment at 25° C. the average skin temperature of lightly clothed men is 33·0° C. (Burton, 1935). In a group of fifty normal subjects, Eddy & Taylor (1931) found that the skin temperature on the back of the forearm averaged 32·75° C. The subjects were stripped at a room temperature of 20° C., and the skin temperature readings were made 10 min. after stripping. Hardy, Milhorat & Du Bois (1941) state that the skin temperature of the clothed forearm remains constant at 33·8° C. at environmental temperatures of 22–29° C.

Buchthal, Høncke & Lindhard (1944) recorded the temperatures of different muscles. They found that the temperature in the brachioradialis muscle was approximately 35° C., and might be much lower. Their results differ considerably from our own. This may be explained by their use of a shorter needle thermocouple (18 mm. as against 25 mm.). There is a gradient of temperature in the forearm from the deep tissues to the skin, so at a depth of 18 mm. the temperature will be lower than at a depth of 25 mm.

It is not stated by Buchthal *et al.* how long after exposure the muscle temperature was recorded, although it is noted that cooling takes place on exposure.

The figures for skin, subcutaneous tissue, deep-muscle and mouth temperatures, which were obtained immediately after baring the forearm, have been examined for possible correlations. Between all four temperatures there is a degree of possible correlation which is significant between those of skin and subcutaneous tissue, but between those of subcutaneous tissue and deep muscle the significance is not on such a high level. There is only a low level of significance in the correlation between skin and deep-muscle temperatures. Mouth temperature, being largely invariant, does not show a significant correlation with the temperature of the forearm tissues. Hardy *et al.* (1941) found that skin temperature changes were not correlated with rectal temperature.

The average blood flows in the forearm at different water-bath temperatures are shown in Table 2. They were calculated from the results obtained by Barcroft & Edholm (1943) and from other results obtained subsequently. The

blood flow in the forearm covered with cotton-wool (3·1 c.c.) is rather less than in the forearm in water at 34·0° C. but definitely greater than the blood flow in the forearm in water at 33·0° C. Table 2 also shows the temperature of the skin, subcutaneous tissue and deep muscle of the forearm immediately after baring and in water at different temperatures. The temperature of the skin was assumed to be the same as the water temperature. The tissue temperatures were taken after a steady state had been reached, the time for this varying with the water-bath temperature (see Fig. 2).

The standard or 'normal' conditions are considered to be those conditions existing in the adequately clothed forearm at rest. The term 'adequately clothed' is used to mean that the covering of the arm is sufficient to maintain a comfortable constant temperature. The conditions in the clothed arm are considered to be closely similar to those found in the forearm immediately after baring. The time interval between baring and obtaining the first temperature readings was sufficiently short to ensure that any cooling that might have occurred would not be significant. This is confirmed by extrapolating the curves obtained for the rate of cooling of the forearm on exposure to room temperature (see Fig. 1). The average error was less than 0·1° C. The state of the forearm covered with cotton-wool is also similar to that of the clothed forearm. The skin and deep-muscle temperatures remained nearly constant over a period of 2 hr. in the covered forearm, and the initial readings were identical with those obtained from the group of normally clothed individuals immediately after baring the arm. It is considered, therefore, that the rate of blood flow in the forearm covered with cotton-wool must be nearly the same as that in the adequately clothed forearm at rest.

These data enable a choice to be made of a water-bath temperature which will ensure the least alteration of forearm conditions from those existing in the clothed forearm. It is not possible to select a water-bath temperature which will not result in some alteration in these conditions. If a water temperature of 35·0° C. is used, the deep-muscle temperature remains constant at the standard level, but both skin and subcutaneous temperatures rise. To keep these last two temperatures constant a bath temperature of 33·0° C. must be used, but this will result in a substantial fall of deep-muscle temperature. Similar conclusions are reached if the rate of blood flow is used as a criterion. At a water-bath temperature of 35·0° C. the blood flow is considerably greater than in the forearm covered with cotton-wool; at 33·0° C. the flow is considerably less. Examination of the conditions existing in the intermediate range shows that there is less net alteration in temperature and that the blood flow is of the same order as that in the covered arm when the forearm is immersed in water at 34·0° C.

What are the advantages of using such a bath temperature apart from the fact that conditions will be as near as can be obtained to those existing in the

clothed forearm? Data obtained by using other temperatures may be misleading. Abramson & Fierst (1942) used a temperature of 32·0° C. for their water-bath and found the average blood flow in the forearm to be 1·7 c.c./100 c.c. forearm/min. Pickering (1943) used this figure to calculate the total blood flow to the musculature of the body, assuming the forearm blood flow to represent muscle blood flow. This gave a figure of 500 c.c./min. For the reasons given above, however, the blood flow in a forearm in water at 32·0° C. is not representative of the conditions existing in the clothed body, and the total muscle blood flow as calculated is too low. If the figure of 3·1 c.c., representing the blood flow in the covered arm, had been used for the calculation the total muscle flow would be 900 c.c./min.

Other workers have used lower water-bath temperatures. The use of low temperatures may result in the masking of the response of the forearm blood vessels, owing to their constriction. This is illustrated by the experiment described above in which the degree of increase in forearm blood flow, provoked by placing the feet in hot water, is only slight when the forearm is in water at 30·0° C., is definitely increased when the water-bath is at 32·0° C., and the effect is considerable when the arm water-bath is at 35·0° C. This experiment may explain the original failure of Grant & Pearson (1938) to demonstrate any effect on forearm blood flow when heat was applied elsewhere to the body. They used a water-bath at a temperature of 30·0° C., and before the experiment the subject was kept stripped in a room temperature of 15° C. All forearm temperatures fall when the arm is exposed (see Fig. 1), and the tissues would be kept at a low temperature by such a cold water-bath. The constricted forearm vessels would only react to the strongest stimuli. In fact, Grant & Holling (1938) subsequently reported that they had obtained a definite increase in the forearm blood flow, but this required what may be described as heroic heating.

Temperatures higher than 35·0° C. may also be undesirable, as it has been shown that fluctuations in the rate of blood flow increase with higher water-bath temperatures. In particular, the major fluctuation known as the 'die away' effect is very noticeable at temperatures above 37·0° C. It is concluded that the most satisfactory water-bath temperature to select is one between 33·0 and 35·0° C.

The fact that the arm cools when it is bared is of some practical importance. The use of the plethysmograph for measuring blood flow inevitably means some delay between the initial exposure of the forearm and the final adjustment of the apparatus. When the first blood-flow measurements are made, temperatures in all portions of the forearm may have fallen considerably, so the blood flow will be reduced.

Grant & Pearson (1938) have previously recorded the rate of cooling of the forearm in one subject. In 75 min. the skin and deep-muscle temperatures

fell 5·5 and 4·7° C. respectively. In only one of our five subjects was the fall so steep; this may be because our room temperature was slightly higher (18·5° as compared with 15·0° C.). Freeman & Nickerson (1938) recorded the changes in skin temperature at ten different areas of the body, the subjects being nude. After 2 hr. the average forearm skin temperatures had fallen to 30·8° C. at a room temperature of 20·0° C.; when the room temperature was 15·0° C., after 2 hr. the skin temperature averaged 29·0° C. These results suggest that, when the whole body is exposed, the rate of cooling of any given area may be less than if that part is the only one exposed. Indeed, Eddy & Taylor (1931) measured skin temperatures in a group of five normal subjects stripped in a room at a temperature of 20·0° C. and found no significant change after 1 hr. exposure.

Lewis (1927) measured changes in subcutaneous temperature when the arm was placed in water, and found that a steady state was reached within 5 min., irrespective of the bath temperature. It will be seen from Fig. 2 that the subcutaneous temperature may take up to 2 hr. to reach a steady state. These results amplify those previously reported (Barcroft & Edholm, 1943) and emphasize that exposure of the forearm to low and high temperatures produces alteration of the temperature of all tissues. Many authors still conclude, following Lefevre (1911), that such temperature changes are confined to the superficial tissues and do not affect the deeper levels.

SUMMARY

1. The temperatures of the skin, subcutaneous tissue and deep muscle measured in the upper part of the resting forearm immediately after baring were 33·0, 33·6 and 36·2° C. respectively.

2. The average blood flow in the resting covered forearm is 3·1 c.c./100 c.c. forearm/min.

3. These figures are compared with those for the forearm in water at different temperatures.

4. Although it is not possible exactly to reproduce the conditions in the adequately clothed forearm by immersion in water, a water-bath temperature of 34·0° C. produces less alteration in these conditions than any other bath temperature.

5. The use of this water-bath temperature during plethysmographic determinations of the forearm blood flow has some advantages; they are discussed.

6. The various forearm temperatures were measured at short intervals for 2 hr. while the forearm was: (i) Uncovered and exposed to air. The final temperature readings were, skin 27·9° C., subcutaneous layer 28·5° C., deep muscle 30·7° C. (ii) Immersed in water. The final subcutaneous temperatures

ranged from 14·6° C. (water-bath, 12° C.) to 38·7° C. (water-bath, 41° C.). For the deep-muscle layer the corresponding figures were 18·6 and 37·8° C. respectively.

Thanks are due to the members of the Belfast Medical Students Association who acted as subjects. We are indebted to Dr Q. H. Gibson for assistance with the statistical calculations.

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THE EFFECT OF MAGNESIUM DEFICIENCY ON NEURO-MUSCULAR TRANSMISSION IN THE SHORE CRAB, *CARCINUS MAENAS*

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The transmission of excitation at the neuro-muscular junction of the walking leg of *Carcinus maenas* is characterized by the existence of a natural 'block', which can be overcome by the summation of nerve impulses arriving there (Pantin, 1936). A just visible slow contraction of the flexor muscle of the dactylopodite is observed when impulses arrive at the neuro-muscular junction at a frequency of about 10 impulses/sec., whereas a maximal contraction is approached at a frequency of about 300 impulses/sec.

The way in which excitation is transmitted from nerve to muscle in *Carcinus* is not understood, but investigation of the effects of ions and drugs on transmission promises to throw considerable light on the nature of the mechanisms involved. One of the most striking of the few positive facts yet discovered is the capacity of the magnesium ion to 'curarize' the junction.

According to Katz (1936*b*), perfusion of the crab's limb with a fluid containing 1·5–2 times the blood concentration of magnesium depresses neuro-muscular transmission, so that higher frequencies of stimulation must be applied to the nerve in order to call the majority of muscle fibres into action. At 2·5 times the blood concentration, magnesium produces a complete 'curarization', which is, however, reversible (Katz, 1936*a*). In order to interpret the effect of excess magnesium Katz (1936*a*) suggested that 'It would be simpler to invoke a chemical transmission of some kind [between nerve and muscle], and to suppose that the transmitter either is not formed, or is destroyed more rapidly, under the influence of Mg'.

It was with the object of extending the evidence bearing on Katz's suggestion that the following experiments on the effect of Mg deficiency on neuro-muscular transmission were carried out.

METHODS

The method of preparing and treating the walking leg of the crab was very similar to that described by Pantin (1934, 1936) and therefore needs no elaborate description. The leg was removed and attached to a rubber tube connected with bottles for perfusion, the perfusion apparatus being

arranged so that the fluid flowing through the limb could be rapidly changed. Silver-wire electrodes were carefully hooked through the arthrodial membranes of the limb. The limb was set up for recording the flexion of the dactylopodite by attachment of its tip to a spring lever writing on a smoked drum.

Stimulation of the nerve was effected by groups of condenser discharges through a neon lamp. The frequency of discharge was varied by inserting in the charge circuit a resistance, the effect of which had been calibrated by an oscillograph. The intensity of individual discharges could be varied by means of a potentiometer in the discharge circuit. By means of a short-circuiting device in the discharge circuit, groups of discharges could be applied to the nerve for measured periods of time.

Since the application of continuous current above threshold strength to the crab's nerve is liable to generate more than one nerve impulse (Jasper & Monnier, 1933; Katz, 1936*b*), it is important that each condenser discharge should be of shorter duration than the absolute refractory period of the nerve to be stimulated. The absolute refractory state in *Carcinus* nerve lasts for about 1 msec. (Pantin, 1936). The duration of the condenser discharges employed in these experiments was of the order of 0·05 msec.

The preparation was perfused with fluids containing various concentrations of Mg, made up for the most part by the mixture of two stock solutions. The 'blood-Mg' stock solution contained the ions of Na, K, Ca and Mg in the same concentration as was found by analysis in *Carcinus* blood by Bethe (1929): Na 11·3 g./l., K 0·56 g./l., Ca 0·56 g./l., Mg 0·64 g./l. The whole stock solution was brought to *p*H 7 by the addition of $NaHCO_3$ dissolved in a portion of the solution. The second, or 'Mg-free', stock solution contained no Mg salt, but otherwise resembled the blood-Mg solution in composition. From these two stock solutions various mixtures were made up for perfusion and will be referred to in the text in terms of the percentage of the blood concentration of Mg they contained, e.g. the '80% Mg' solution.

There is some objection to the use of a Mg-free stock solution which is not isotonic with the blood-Mg solution. In order to meet this objection a third stock solution was used on occasions. This was also free of Mg, but was made isotonic with the blood-Mg solution by the addition of the appropriate quantity of NaCl. The use of the isotonic Mg-free solution gave results no different from those obtained with the slightly anisotonic Mg-free solution.

It is probably incorrect to assume that all the Mg present in *Carcinus* blood is in ionic form. Although no estimation of the amount of un-ionized Mg present in *Carcinus* blood is available, some indication of the amount to be expected can be obtained from Robertson's (1939) figures for *Cancer* blood. The blood of *C. pagurus* contains 0·658 mg. Mg/g. blood, a small quantity of which is present in undialysable form. The amount of ionic Mg exceeds 0·6 mg./g. blood, or 90% of the total blood concentration. It is unlikely that in *Carcinus* the proportion of un-ionized Mg greatly differs from that in *Cancer*. In any event, it is exceedingly unlikely that the proportion of ionic Mg falls below 44% of the total. Yet, in a series of experiments to be described, results were obtained by reducing the Mg content of the perfusion fluid from 44 to 9% of the blood concentration which were similar to those obtained when the Mg concentration was lowered to 10% of the blood value. It can safely be assumed that the results which are now described are not due to a failure to make allowances for the un-ionized Mg included in Bethe's analysis of crab's blood.

RESULTS

The limits of magnesium deficiency

Experiments were conducted in order to establish the lowest limit of Mg deficiency which the crab's nerve-muscle preparation could survive unimpaired. It was found that the preparation was perfectly viable when perfused with concentrations as low as 0·032 g./l. Mg (5% blood concentration).

Perfusion with concentrations of 3% blood-Mg or lower, however, first impaired the response of the muscle to stimulation of the motor nerve and finally abolished it altogether. Impairment of the response, as can be seen in Fig. 1, consisted both in reduction of the tension of the contraction, particularly at lower frequencies of stimulation, and in prolongation of the phases of contraction and relaxation. After disappearance of the response a return to higher concentrations of Mg did not restore the normal behaviour.

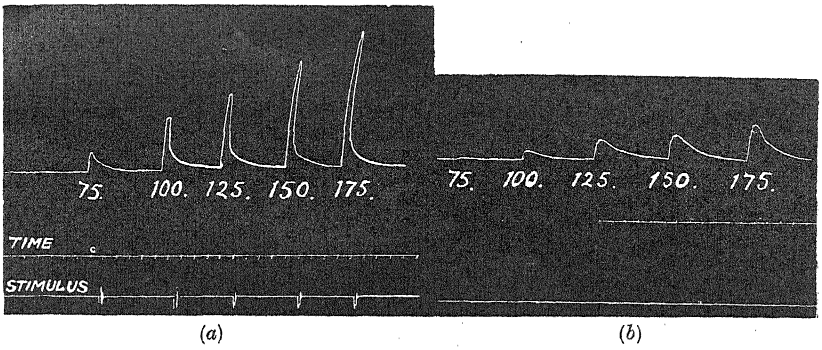


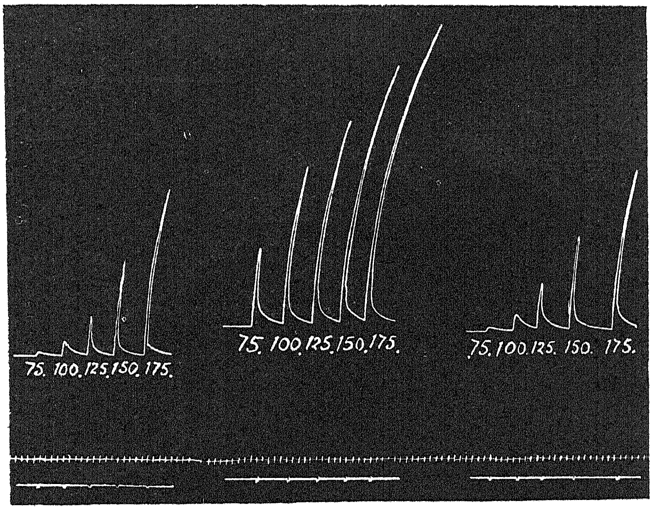
Fig. 1. Responses of muscle to stimulation of the motor nerve at frequencies of 75, 100, 125, 150 and 175 shocks/sec. Stimulation lasts in each case 0·2 sec. Time in sec. (*a*) Perfused with fluid containing 44% blood-Mg. (*b*) 20 min. after beginning perfusion with Mg-free fluid.

Magnesium deficiency and the muscular response

If a sufficient number of shocks are applied to the motor nerve at a sufficiently high frequency, the tension of the muscular response reaches a maximum. The magnitude of the maximum tension is not greatly affected by variations of the Mg content of the perfusion fluid between the blood value and 5% of it. If stimulation of the nerve, however, is restricted to short groups of shocks, maximal contractions are not necessarily evoked from the muscle. It is largely with submaximal tensions, obtained after brief stimulation of the motor nerve, that the experiments described in this and the subsequent section of this paper deal.

The effect of reducing the Mg content of the perfusion fluid within the limits of the viability of the preparation was investigated as follows. The limb was perfused with a fluid of high Mg content (either blood-Mg, 80% Mg or 44% Mg). The tensions developed by the flexor muscle of the dactylopodite in response to stimulation of the nerve for a definite period of time (0·2–0·6 sec.) at frequencies of 75, 100, 125, 150 and 175 shocks/sec. were recorded. The perfusion fluid was then changed for one of lower Mg content. Tensions obtained by stimulation for the same duration of time at the same series of frequencies were recorded at various time intervals after the change had been

made. Finally, in the majority of cases, the limb was perfused with fluid of the original Mg content, and again records of the muscular response were taken. In these experiments it was regularly observed that, on changing from a high concentration of Mg to a lower, the submaximal muscular response at all frequencies of stimulation was enhanced. On changing from low Mg to higher, on the other hand, a decline in the tension of the muscular response was observed. A typical experiment is illustrated in Fig. 2.



(a) (b) (c)

Fig. 2. Responses of muscle to stimulation of the motor nerve at frequencies of 75, 100, 125, 150 and 175 shocks/sec. Stimulation lasts in each case 0·4 sec. Time in sec. (*a*) Perfused with fluid containing Mg at blood concentration. (*b*) 9 min. after beginning perfusion with fluid containing 10% blood-Mg. (*c*) 3 min. after return to original perfusion fluid.

Table 1 contains a record of some of the experiments performed. The tensions developed by the flexor muscle of the dactylopodite are expressed in

TABLE 1. Tensions (in mm.) obtained by stimulation of the motor nerve during perfusion with various concentrations of magnesium

	Magnesium: percentages of blood concentration								
	Initial perfusion fluid			Second perfusion fluid			Final perfusion fluid		
Exp. no.	100%	80%	44%	60%	20%	10%	100%	80%	44%
1	28	—	—	28	—	—	—	—	—
3	3	—	—	—	9	—	3	—	—
5	3	—	—	—	—	33	3	—	—
7	—	24	—	—	—	53	—	24	—
9	—	5	—	—	—	11	—	2	—
11	—	14	—	—	—	26	—	11	—
15	—	—	5	—	10	—	—	—	2

terms of the mm. moved by the tip of the spring lever. The contractions measured are in all cases those obtained by stimulation of the nerve for a definite period of time at 100 shocks/sec. It will be noted from Table 1 that the greatest effect is seen with concentrations of Mg near the lower limit of viability of the preparation, while less or no effect is observed when the second perfusion fluid contains a relatively high concentration of Mg.

On proceeding from a lower Mg concentration to a higher the tension of the submaximal response is diminished.

Magnesium deficiency and junctional transmission

In the crab's limb a few motor nerve fibres, by repeated branching, supply a large number of muscle fibres. There is good reason for supposing (Pantin, 1936) that the excitabilities of the individual muscle fibres differ, and that a

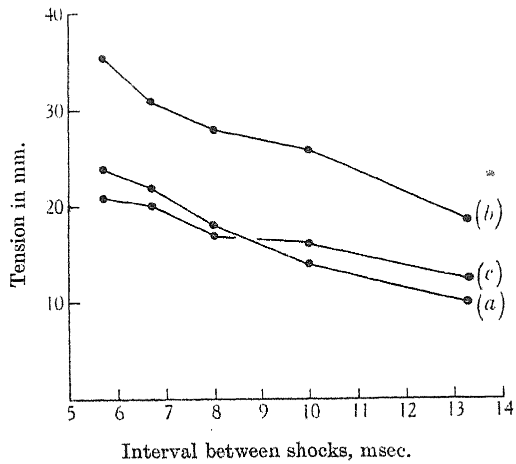


Fig. 3. Isometric tension developed after 50 shocks delivered at given frequencies. (*a*) Perfused with fluid containing 80% blood-Mg. (*b*) 9 min. after beginning perfusion with fluid containing 10% blood-Mg. (*c*) 7 min. after return to fluid containing 80% blood-Mg.

volley of impulses in the nerve, which produces a submaximal muscular contraction, will excite some but not all of the muscle fibres. With submaximal contractions the tension of the muscular response can be taken as an index of the number of muscle fibres to which excitation in the nerve has been transmitted. Pantin has shown that, if precautions are taken to ensure that a single stimulus to the nerve excites only one nerve impulse, then the tension of the muscular contraction resulting from the application of a group of a few shocks to the nerve will depend upon: (1) the time interval between each shock, and (2) the absolute number of shocks. These results give us a means of estimating, from the tension of the muscular response, the extent to which excitation is transmitted from nerve to muscle.

To investigate the effect of Mg deficiency on neuro-muscular transmission two series of experiments were carried out. In one the absolute number of shocks applied to the nerve was kept constant, while the shocks were delivered at various frequencies. In the second, the frequency at which discharges were delivered was kept constant, but various numbers of discharges were applied. As a result of these experiments it was found that neuro-muscular transmission was considerably improved when the limb was perfused with fluids deficient in Mg. The limb perfused with 10 or 5% Mg, as compared with the limb perfused with blood-Mg or 80% Mg, required in order to evoke a given tension of muscular response: (1) a lower frequency of condenser discharge if the number of discharges was kept constant (Fig. 3), and (2) fewer discharges if the frequency of discharge was kept constant (Fig. 4). It is clear that the im-

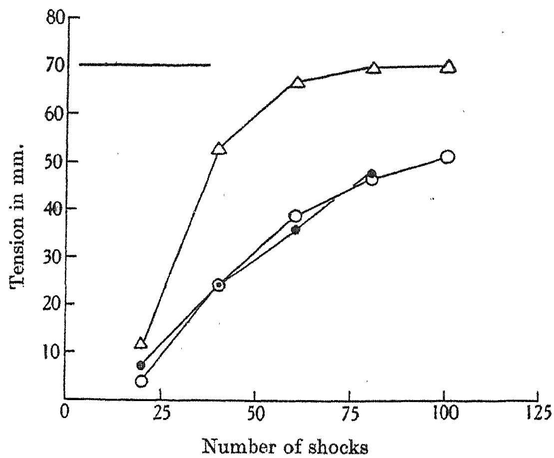


Fig. 4. Isometric tension developed after a given number of shocks at a frequency of 100 shocks/sec. •—• Perfused with fluid containing 80% blood-Mg. △—△ 7 min. after beginning perfusion with fluid containing 10% blood-Mg. ○—○ 8 min. after return to fluid containing 80% blood-Mg.

provement of neuro-muscular transmission due to reduction in the Mg content of the perfusion fluid was lost on reperfusing with fluids containing Mg at or near the blood concentration.

DISCUSSION

The enhancement of the submaximal muscular contractions caused by lowering the Mg content of the perfusion fluid cannot be due to an improvement in the physical powers of shortening of the individual muscle fibres, since the effect is not readily seen if the muscle is made to exert a maximum tension, while it is clearly seen when low frequencies of stimulation are applied to the nerve. Nor can the enhancement of response be due to the production of more than one nerve impulse by each condenser discharge, since the duration of the

stimulus is 1/20th of the absolute refractory period of the nerve. The effect of Mg deficiency can best be explained by supposing that the Mg ion at or near blood concentrations has a depressing effect on neuro-muscular transmission, and that transmission is facilitated by reduction in the amount of Mg ion present. The marked degree of 'block' between nerve and muscle in the shore crab is definitely related to the naturally high magnesium content of the blood.

The evidence on which the above supposition is based can certainly be used to support Katz's hypothesis that transmission between nerve and muscle is effected by a substance, in whose destruction Mg is a co-enzyme or whose production is inhibited by Mg. It is possible, however, to explain the effect of Mg deficiency not in terms of increasing the available amount of a chemical transmitter, but in terms of increasing the excitability of the muscle fibres to an unchanged level of excitation.

SUMMARY

1. Perfusion fluids containing less than 0·032 g./l. Mg (5% blood concentration) first impair and finally abolish the response of the flexor muscle of the dactylopodite of the crab's walking leg to stimulation of the motor nerve.

2. Responses of the muscle to stimulation of the nerve are retained or enhanced during perfusion with fluids containing 5% or more of the blood concentration of Mg.

3. Magnesium deficiency—especially between 5 and 20% blood concentration—enhances the *submaximal* tensions obtainable from the flexor muscle in response to stimulation of the motor nerve. The effect is reversible. The enhancement of submaximal muscular response is due to facilitation of neuromuscular transmission.

4. A supportable Mg deficiency does not greatly enhance the maximum tension of the muscular contraction.

We wish to express our thanks to Prof. H. S. Raper for his advice and to Dr W. Schlapp for his help in calibrating the neon lamp apparatus. The cost of some of the apparatus used was defrayed by a grant to one of us (H. O. J. Collier) by the Government Grant Committee of the Royal Society.

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THE NORMAL VISUAL (ROD) FIELD OF THE DARK-ADAPTED EYE

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The visual field of the dark-adapted eye or rod field has recently been investigated (Livingston, 1944 *a*, *b*) with some thoroughness within a circle of 30° radius from the fixation point, but no data exist with reference to its outer limits. It was therefore decided to examine the size of the rod field in a series of presumably healthy adults by means of a modified Lister perimeter which permitted readings to be taken up to 90° in all directions outward from the fixation point. This would establish a standard which might then be used to detect certain eye diseases in an early stage and would also supplement the work already done within the 30° limits.

METHODS

Apparatus. The Lister perimeter used was of $\frac{1}{3}$ m. radius. A self-luminous radium sulphide point of known candle-power, calibrated at the National Physical Laboratory, was used for the movable target. Stiles (1944) has described the method of measuring the candle-power of such faint test objects. The target found most suitable had a candle-power of $1{\cdot}5 \times 10^{-9}$. Under the conditions of the test, i.e. at a distance of $\frac{1}{3}$ m. from the eye, this stimulus was below the cone threshold. In fact, if the subject looked directly at the radium point so that the image fell on the rod-free fovea the point became invisible. The central fixation point was a white disk ($1\frac{1}{2}$ mm. diameter) as supplied originally with the perimeter for the usual tests with a light-adapted eye, but illuminated by a dim red light the intensity of which could be controlled by a rheostat. The disk subtended an angle of $\frac{1}{4}$° at the eye. A red light was used for illuminating the fixation point, since the cone threshold for red light (particularly for the far red beyond 650 mμ) is relatively little above the rod threshold and therefore foveal (cone) fixation is permitted with least interference to the rod dark-adaptation. In the darkness during the test nothing but the red fixation point and the movable radium point could be seen by the subject.

The Lister perimeter was equipped with the usual means of mapping out the field mechanically on a chart. Although the instrument was designed for use in the light it proved eminently suitable for work in the dark without any alterations, except the necessary provision of the red fixation point and the movable radium target mentioned.

Test procedure. Each subject was dark-adapted before the test for a total period of 1 hr. During the first half-hour he wore R.A.F. red goggles, as used by night flyers for dark-adaptation before operations, and then sat in complete darkness for the second half-hour. Since the test itself occupied from 40 to 60 min., it was desirable to ensure that the dark-adaptation was as thorough as practicable in order to avoid appreciable change during the test. A check on this was made

in practice by taking a final perimeter reading on the same meridian as the first. It should be added that no attempt was made to have the subjects uniformly light-adapted before beginning dark-adaptation. For instance, some came into the test room out of the sunlight and others were tested at night. Also, while wearing the red goggles some sat in a relatively dim room while others moved from room to room, for example, or read at an open window. None, however, went out-of-doors during the period. In practice this procedure appeared satisfactory, as no significant change of dark-adaptation was found during the test itself.

Only one eye of each subject was tested. The eye selected was the 'better' eye of the two. Other conditions being similar, it was the one having the least refractive error. If no great difference existed, then the master eye was selected. No subject wore spectacles during the test. Certain additional tests were made on one subject in an attempt to ascertain the effect of refractive errors on the results generally. These tests are reported below. Tests were made with the natural pupil in all cases. In all positions the subject's head was turned (while keeping the chin in the chin-rest) so that the area of the retina tested was as great as possible and the effect of physical obstruction of the field by the nose, eyebrow, etc., was reduced to a minimum. For the upper part of the plotted field the eyelid was raised. The resulting field is sometimes termed 'absolute'.

The brightness of the red fixation point was kept as low as possible consistent with reasonable ease in fixating. The adjustment was usually made by the operator, but sometimes by the subject. It was found advisable to allow periods of rest from fixation after each few points had been plotted. These periods were more frequently necessary in the initial stages of the test. Subjects varied widely in their ability to fixate for appreciable periods without strain. Of course, it was not possible for the operator to watch the subject's pupil for movement, as can be done in the usual light-adapted perimeter test, and some variability in the results is probably due to imperfect fixation. But it is not believed that this is great. The blind spot was first plotted, since this procedure with its more exacting demands on fixation prepares or trains the subject in some degree for the peripheral test, and, also, enables the operator to judge each subject's control of fixation.

The procedure used in plotting each point on the periphery of the field was to record (mechanically on the chart) the point of disappearance of the radium target as it was moved outward and then the point of reappearance as it was moved inward again, the target being moved at a steady speed in each case. This was done twice for each meridian, giving four points. The limit of the field on the meridian was taken to be half-way between the mid-point of the two outer points of disappearance and that of the two inner points of reappearance. The field was then plotted by joining up the boundary limits thus found. Readings were usually taken on meridians at 15° intervals round the circle.

This method should tend to neutralize the reaction time of both the subject and the operator. Subjects generally found it easier to judge the point of reappearance than that of disappearance. The angular difference between the two points of appearance and disappearance varied widely and was usually 20° and sometimes 40° or more.

Because of the 90° radius limitation of the perimeter used, it was found (particularly on the lower temporal side) that the radium target could be moved out to its limits on the arc before the subject signalled its disappearance. In some such cases the subject found that the target disappeared immediately it ceased to move, due either to cessation of the movement or to the coincidence of the actual limit of the visual field with the physical limit of the target motion. In these cases, when the target was moved inward again, the subject signalled its reappearance only after it had moved some appreciable distance. For a few subjects with very good vision and on one or two meridians the target failed to disappear at all when moved outward to its limiting position: only on these meridians and for these subjects has the field been drawn as reaching the 90° circle.

The blind spot could not be plotted with any great accuracy since the width of its projected image on the chart was comparable to the error in determining its boundary by moving the radium point from the region of visibility to that of invisibility or the reverse. However, by means of a somewhat different technique from that described above for the peripheral boundary,

the blind spot was plotted as accurately as possible in all cases. It should be made clear here that the experiment was designed to plot the outer limits of the field and relatively little attention was given to abnormalities within the outer boundaries of the field, though, in some cases, areas of reduced sensitivity were noted.

In order to provide a single figure indicative of the size of a field, the mean angular radius (i.e. angular distance from the centre of the plotted field) was calculated as an average of the plotted values on the twenty-four meridians at 15° apart.

Subjects. Forty-seven subjects were used for the tests. They were mostly University students and hospital and laboratory staff, but others were included. All pathological cases or other cases of known or suspected abnormal vision were avoided with the exception of those having errors of refraction only. Subjects who gave suspiciously small or abnormally shaped fields were particularly examined for retinal abnormalities and were also tested (with the kind co-operation of the Oxford Nutrition Survey) for vitamin A content of the blood. If no abnormalities were detected and no vitamin A deficiency reported, the results were accepted.

RESULTS

The results for all subjects tested are given in Table 1. Thirteen of the subjects were tested twice or more to ascertain the magnitude of individual variation from test to test. The average result is given for each of these. The individual values on which these averages are based are given in Table 2. Subjects have been classified into age groups.

It will be seen from Table 1 that the average value for the twenty-three subjects in the 10–20 age group is a mean radius of field of 59°. For the 20–30 age group, in which there were eleven subjects, the average is 64°. The other (higher) age groups contain only a few subjects each, and the averages have therefore not much significance. (It is hoped to submit results of tests on more subjects in these groups later.) It will be observed, however, that there appears to be a tendency for the size of field to become less with increasing age. The range of individual results in all groups is large. It may be mentioned here that the extreme values shown at the foot of Table 1 are those from the results of single tests on individual subjects, and the results of the first tests in the case of subjects tested more than once in the same age group.

As the subjects in the 10–20 and 20–30 age groups together number thirty-four and form the majority of those tested, it may be of interest to consider them also as one combined group. Actually, there were no subjects in the 10–20 group under 17 years of age, so that the combined group is virtually one of 17–30 years. As will be seen from Table 1 the average result for this combined group is 61°, the extreme values being 50 and 71°. A frequency diagram for this combined group is shown in Fig. 1. The spread is considerable and is similar to the spread found by other observers on the final rod thresholds measured by dark-adaptometer at one retinal location. Compare, for instance, the results of Hecht & Mandelbaum (1939) on 110 normal persons.

Referring to the individual results of repeated tests as shown in Table 2, it will be seen that the difference from test to test for any one subject is small in about half the cases and does not exceed 9° in any one case (subject No. 45).

TABLE 1. Mean angular radius of rod field in degrees using radium target of $1{\cdot}5 \times 10^{-9}$ c.p.

Subject no.	Sex	Age group (years) 10–20	20–30	30–40	40–50	50–60	60–70
			Single tests				
1	F.	50	—	—	—	—	—
2	F.	—	65	—	—	—	—
3	F.	57	—	—	—	—	—
4	F.	—	62	—	—	—	—
5	F.	—	56	—	—	—	—
6	M.	61	—	—	—	—	—
7	M.	63	—	—	—	—	—
8	M.	63	—	—	—	—	—
9	M.	62	—	—	—	—	—
10	F.	—	69	—	—	—	—
11	M.	58	—	—	—	—	—
12	F.	61	—	—	—	—	—
13	M.	—	68	—	—	—	—
14	M.	50	—	—	—	—	—
15	F.	—	64	—	—	—	—
16	M.	58	—	—	—	—	—
17	M.	63	—	—	—	—	—
18	M.	57	—	—	—	—	—
19	M.	54	—	—	—	—	—
20	M.	53	—	—	—	—	—
21	F.	60	—	—	—	—	—
22	F.	—	—	63	—	—	—
23	F.	60	—	—	—	—	—
24	F.	63	—	—	—	—	—
25	F.	50	—	—	—	—	—
26	F.	65	—	—	—	—	—
27	F.	—	—	—	—	55	—
28	F.	64	—	—	—	—	—
29	F.	—	—	—	—	52	—
30	M.	—	—	—	—	44	—
31	F.	—	—	—	—	56	—
32	F.	—	—	—	65	—	—
33	M.	—	—	55	—	—	—
34	M.	—	—	—	—	—	40
			Average of repeated tests				
35	F.	—	67	—	—	—	—
36	F.	—	—	68	—	—	—
37	F.	—	—	—	—	63	—
38	F.	—	71	—	—	—	—
39	F.	—	—	59	—	—	—
40	M.	—	—	—	44	—	—
41	F.	—	—	—	61	—	—
42	F.	—	70	—	—	—	—
43	F.	66	—	—	—	—	—
44	M.	—	51	—	—	—	—
45	F.	67	—	—	—	—	—
46	F.	—	60	—	—	—	—
47	F.	63	—	—	—	—	—
No. of subjects in each group		23	11	4	3	5	1
Average mean radius		59	64	61	57	54	40
Extreme values of mean radius		50–71	55–71	55–69	45–65	44–63	—
Average mean radius for 34 subjects in 10–30 combined age groups		61					

The time intervals between successive tests were not uniform and varied from a day or two up to a month or more.

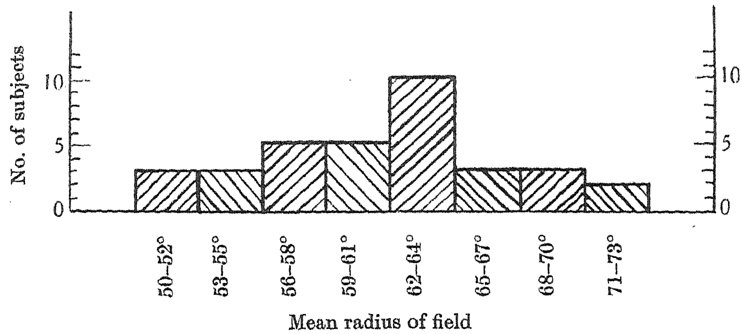

Fig. 1. Frequency diagram showing mean radius of field for thirty-four subjects in the combined age group 10–30 years.

The variation in shape of the fields is of interest. Those of one subject (No. 39) who was tested three times are reproduced (Fig. 2). The mean radius (which is indicated on the respective fields) is 57, 61 and 58°. It will be observed that the fields differ rather considerably in shape. This is typical of repeated tests. A substantial difference of readings on a single meridian can, however, account for a large change in the shape.

TABLE 2. Individual values for repeated tests. Mean angular radius of rod field in degrees using radium target of $1 \cdot 5 \times 10^{-9}$ c.p.

Subject	No. of tests	Results of individual tests	Averages
35	3	65 67 69	67
36	3	69 67 67	68
37	3	63 63 63	63
38	3	71 70 72	71
39	3	57 61 58	59
40	4	45 40 46 45	44
41	3	62 60 61	61
42	3	70 69 70	70
43	3	63 67 69	66
44	3	55 48 49	51
45	2	71 62½	67
46	2	62 58	60
47	3	60 65 64	63

Effect of errors of refraction on results. Since it was not permissible for subjects to wear their spectacles during the test it was of some interest to determine to what extent, if any, the variation of results between individuals was affected by variation in degree and type of refractive correction needed.

For this purpose the effect of variation of refraction was produced artificially by means of lenses. Use was made of contact lenses to avoid the chance of interference with the field by frames or edges of even large spectacles. These lenses were not accurately ground to fit the individual eye, as they would

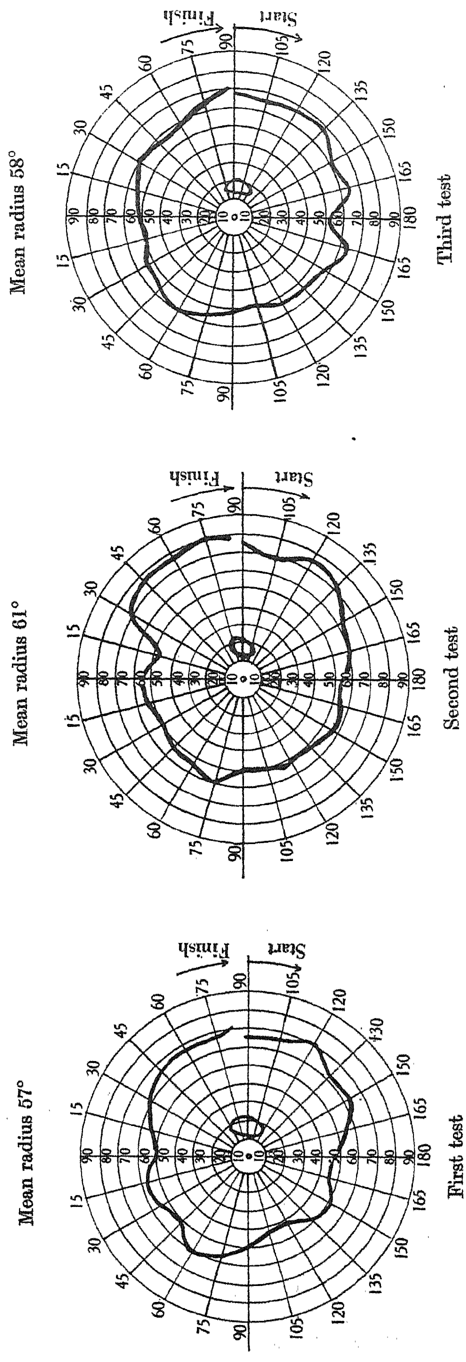

Fig. 2. Rod field for right eye of subject No. 39 in 30–40 age group for target $1 \cdot 5 \times 10^{-9}$ c.p.

normally be for constant wear, but they served the purpose almost as well. They were actually trial contact lenses of known dioptric power.

One subject (No. 39) was chosen for the test and her refraction measured on Snellen's type (in full daylight) with and without contact lenses in position. Without a contact lens the refraction was +2 D. and it was found that with a +6 D. sph. contact lens a −2·75 D. spectacle lens (worn in front of the contact lens in a test frame) was needed to make her emmetropic. With a +4 D. sph. contact lens a −1 D. sph. was needed, and with a plano-contact lens a +1·5 D. spectacle lens was required. The lack of complete uniformity was due to the scleral fit of the trial contact lenses not being equally good in all of them. A dark-adapted rod field test was made on this subject while wearing a plano-contact lens. The lens was then changed (under dim red illumination) for a +4 D. sph. contact lens and a further test made immediately. In both cases readings were taken at 30° intervals only. Finally, a few check readings were taken for the naked eye after removing the contact lens.

With the plano lens the mean radius of the rod field was 62° and with the +4 D. lens it was 63°. The mean of the check measurements made on six meridians for the naked eye was 63°. It may thus be fairly concluded that the results of the rod field test are not significantly affected by low errors of refraction (i.e. between −1 and +2 D.).

Choice of brightness of test object used. As mentioned in the early part of the paper, a test object having a candle-power of $1{\cdot}5 \times 10^{-9}$ was found most suitable and was used throughout. It would have been interesting to have plotted the field for a number of subjects with targets of different brightness. But in the majority of cases the target used gave a large field limited on certain meridians by the 90° physical limits of the perimeter. The use of a brighter test object was not therefore desirable and fainter ones were not available (and would doubtless be difficult to calibrate).

However, in the case of one subject (No. 40), who gave a field considerably below the average with the usual test object, the field was also plotted for a $4{\cdot}5 \times 10^{-9}$ c.p. test object (i.e. one three times brighter). The mean radius of this field was 51°, and that for the fainter (usual) test object, plotted at the same sitting, 36°, i.e. a ratio of 1·4 to 1 in mean radius.

The subject found that he was not only able to see the brighter test object more easily but was also able to judge the points of appearance and disappearance with greater facility and precision. This could be explained on the assumption that the threshold sensitivity of the periphery fell off more rapidly farther out from the fovea.

That the sensitivity may, in fact, fall off in this way can be seen from curves published by Stiles & Crawford (1937) for the dark-adapted retina for two subjects. The curves are considerably different for the two subjects and,

also, for the two meridians tested, but in one case the sensitivity is shown to be almost constant from 20° to about 60° out from the fixation point on the temporal side and then to fall off rapidly.

SUMMARY

1. The rod visual field of the completely dark-adapted eye was mapped out within 90° limits from the fixation point for forty-seven normal individuals by means of a perimeter with a faint radium target below cone threshold.

2. The average values of the mean angular radius of the plotted fields for different age groups using a target of $1{\cdot}5 \times 10^{-9}$ c.p. at a distance of $\frac{1}{3}$ m. from the eye is given.

3. Repeated tests on a number of individuals showed that, while the shape of the field for any individual might vary appreciably from test to test, its area or size as expressed in terms of its mean angular radius was fairly constant.

4. Results are apparently not affected by low errors of refraction.

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THE STIMULATING ACTION OF ACETYLCHOLINE ON THE HEART

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In 1882 Gaskell, when studying the action of the vagus on the heart of the frog, found that the cardiac slowing produced by stimulation of the nerve was commonly followed by an increased activity, and, in some cases, this increase might be the only result seen. Because of the discovery of the sympathetic fibres by Schmiedeberg (1871), Gaskell (1900) was disposed to conclude that the increased action was due to stimulation of sympathetic fibres passing with the vagus. The after-action he considered to be evidence for an anabolic action of the vagus. The use of acetylcholine, which is liberated by the vagus, appeared to offer a method of further investigation of the problem.

The only other experiments in this direction appear to be some by Feldberg & Minz (1931), who, in studying the effects of acetylcholine on ganglia, noted that, after intravenous injection of the drug, there was occasionally an acceleration of the heart *in situ*, even after the vagi had been cut and the stellate ganglia removed.

METHODS

The experiments were carried out on the isolated hearts of cats, rabbits and rats. The coronary arteries were perfused with Ringer-Locke's solution by the usual Langendorf-Locke modification of the Martin method. In several experiments the calcium chloride concentration of the Ringer-Locke solution was lowered from 0·024 to 0·016%. The drugs to be tested were dissolved in the perfusion fluid and injected in a volume of 0·5 c.c. into the tubing close to the heart. In each case the 0·5 c.c. were injected at a standard rate calculated to give an approximately tenfold dilution of the drug by the perfusion fluid. In some experiments the auriculo-ventricular (A.V.) bundle was cut by inserting a narrow knife through the left ventricle between the coronary arteries; auricular and ventricular contractions were then recorded separately. In all tracings the upstroke is due to systole, the downstroke to diastole.

RESULTS

In hearts in good condition the injection of 5μg. acetylcholine causes slowing and weakening of the heart which, however, is almost invariably followed by a period of increased activity (Figs. 1, 2). The stimulating phase sometimes lasts for more than 15 min. During this phase extra systoles are commonly

seen (Fig. 1). In a few hearts the slowing is preceded by a few very forcible contractions. If, in fresh hearts, the injections of 5μg. acetylcholine are

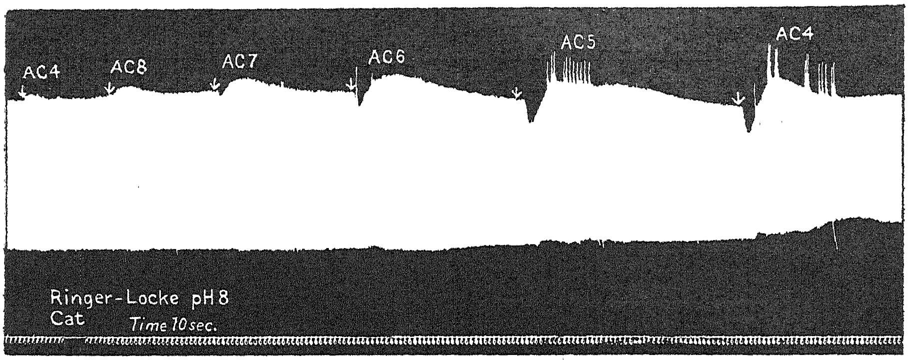

Fig. 1. Cat. The effect of increasing doses of acetylcholine. 0·5 c.c. of AC6 is the equivalent of 0·5μg. and the other dilutions are corresponding multiples of divisions of 10. Note the increasing frequency of extra systoles during the stimulative phases.

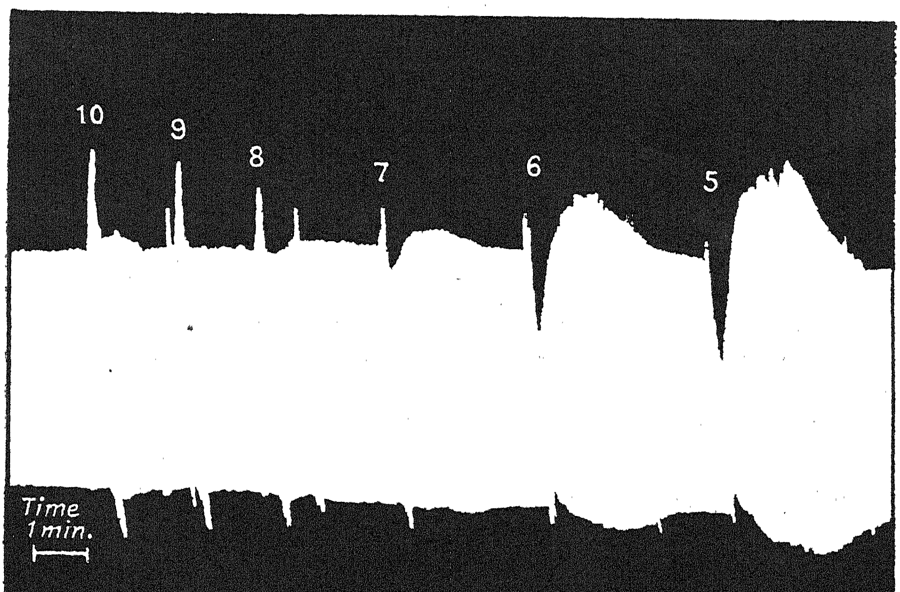

Fig. 2. Cat. The effect of increasing doses of acetylcholine, as in Fig. 1, in an instance in which the initial stimulating effect was particularly well marked. This is an unusual response.

repeated during the stimulating phase of the previous acetylcholine response, the inhibiting effect of acetylcholine gradually diminishes and eventually disappears.

With smaller doses of acetylcholine the response of the heart varies greatly and is dependent on the season. During the months of May and June, when sympathetic activity is known to be good (Armitage, McDowall & Mathur, 1932), small doses of acetylcholine (0·00005–0·05μg.) usually have a stimulating action on the heart; on the other hand, during the winter months such action cannot always be observed with these small doses of acetylcholine. But even in May and June it is essential, in order to demonstrate the stimulating action of small doses of acetylcholine, that the preparation should be from an animal in good condition, fresh and not exhausted from a previous stimulation by adrenaline or high temperature. A further advantage is afforded by the use of the Ringer-Locke solution with the low calcium content (see Methods).

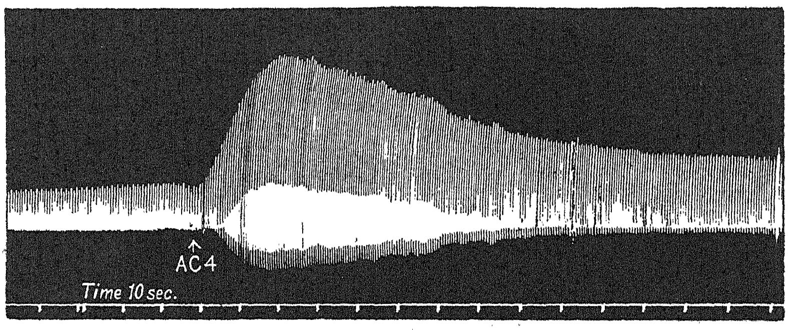

Fig. 3. Cat. The effect of a large dose of acetylcholine (50μg.) after atropine (16·6μg.).

Typical results are shown in Figs. 1 and 2. The smallest effective dose causes stimulation only; with increasing dose the inhibiting effect appears first in the tracing as a slight notch superimposed upon the stimulating effect. It is interesting to note that when the inhibiting action of acetylcholine passes off, and particularly towards the end of the period of increased activity by acetylcholine, the response of the heart to adrenaline is increased. As this appears to be a general phenomenon it is made the subject of a subsequent paper.

After atropine. A dose of 15μg. atropine, which abolishes the inhibiting action of acetylcholine on the heart, has no effect on the stimulating action. After atropine it is possible to discern a stimulating effect of acetylcholine on the force of the contractions and on their frequency. The effect on the force is constant and usually the sole effect. Acceleration occurs only rarely and is best seen at the commencement of an experiment when the preparation is fresh. In the experiment of Fig. 3 the effect of acetylcholine on the atropinized heart is on the force only; during the stimulating phase the heart not only contracts more forcibly in systole, but also relaxes more completely in diastole. This is not always the case. In a number of experiments, especially in summer,

the heart, during the stimulating phase, was seen to beat in a more systolic position. Such an experiment is illustrated in Fig. 4.

Some atropinized hearts show the stimulating effect of acetylcholine better than others under apparently similar conditions, and on repeated injections of acetylcholine some continue to show the stimulating effect much longer than others. In six out of eighty atropinized hearts no stimulating action of acetylcholine could be observed.

With doses of atropine much larger than 15μg., the stimulating effect of acetylcholine on the heart is also affected and sometimes even abolished.

It is possible to unmask the stimulating action of acetylcholine by methylene blue which abolishes the inhibiting action, but this atropine-like action may be abolished by the injection of lactic acid or by stopping the coronary perfusion for a short period.

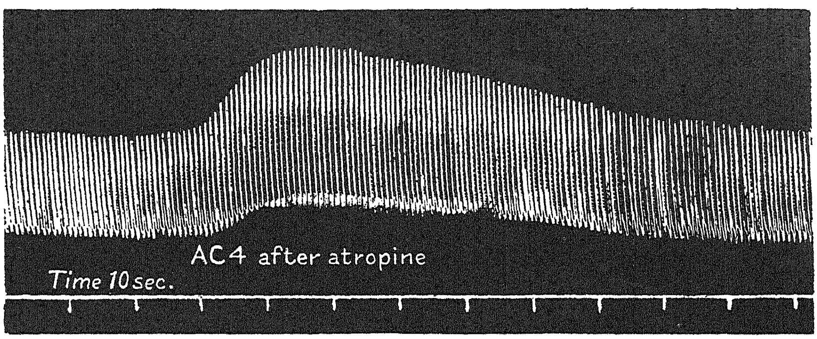

Fig. 4. Cat. The effect of a large dose of acetylcholine (50μg.) after atropine (16·6μg.). Note the increase of tone. This latter only occurs occasionally.

The effects of acetylcholine on the heart are not due to the slight variation in coronary flow produced by acetylcholine. Similar changes in coronary flow produced by other means did not affect the rate or force of the heart.

After eserine the stimulating action of acetylcholine on the atropinized heart is enhanced. The action of acetylcholine may simulate that produced by adrenaline in affecting the rate and the force of the contractions simultaneously (Fig. 5).

After ergotoxine the stimulating action of acetylcholine on the atropinized heart is abolished or may even be reversed (Fig. 6).

Nicotine, in doses which paralyse autonomic ganglia, as indicated by the failure of a second dose to cause slowing, does not abolish the stimulating action of acetylcholine on the atropinized heart. This action, therefore, must be due to an effect of acetylcholine on the heart muscle; it cannot be due to an action on aberrant sympathetic ganglia in the heart itself. The presence of such ganglia is, in fact, somewhat doubtful. The effects of nicotine itself on the heart

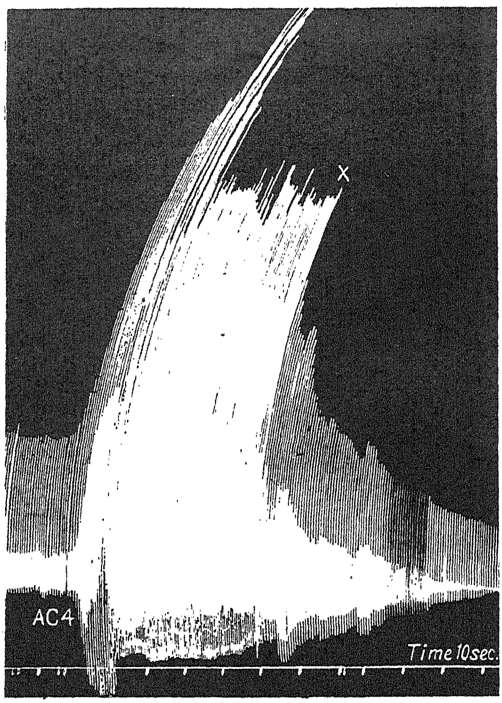

Fig. 5. Rabbit. The effects of a large dose of acetylcholine ($50\mu g.$) after atropine ($16{\cdot}6\mu g.$) and eserine. The drum was stopped at X to facilitate reproduction of the record.

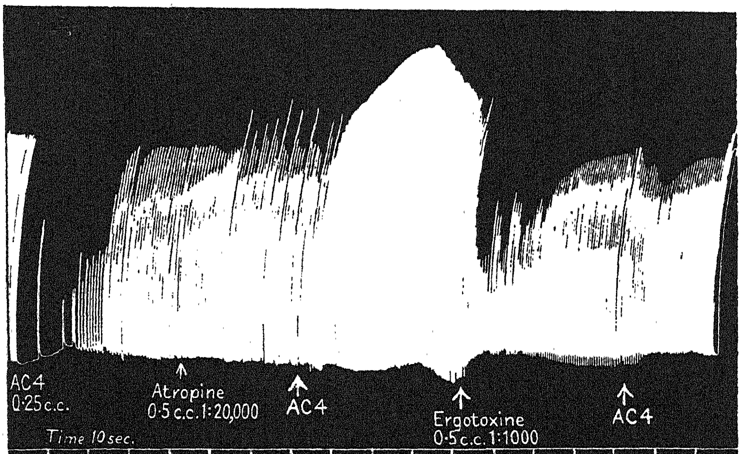

Fig. 6. Cat. At the beginning of the record the effect of a large dose of acetylcholine ($25\mu g.$) is shown, and later the effect of the same dose after atropine ($25\mu g.$). This effect was abolished by an injection of ergotoxine ($500\mu g.$).

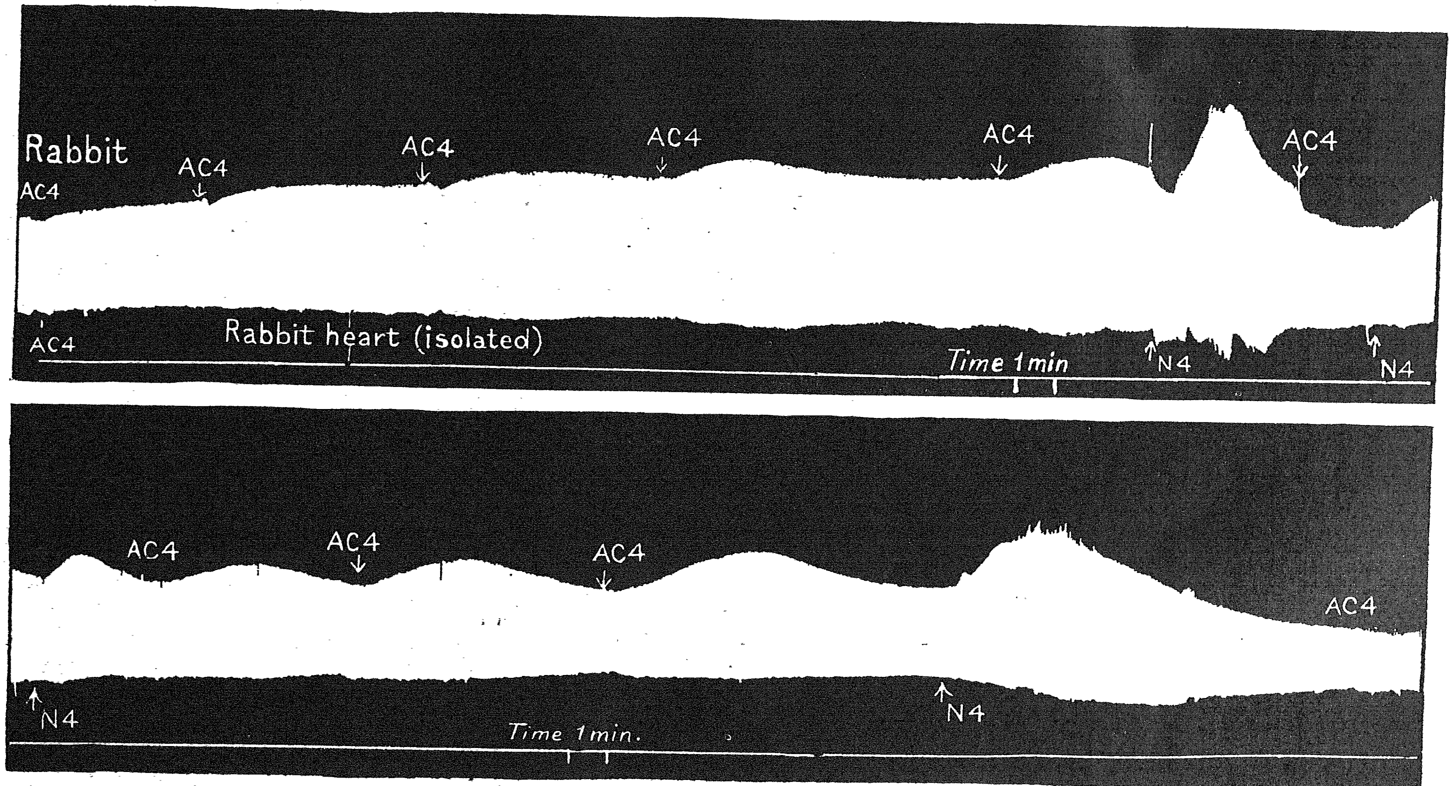

Fig. 7. Rabbit. Showing the effect of acetylcholine (50 $\mu g.$) after a dose of atropine (16·6 $\mu g.$) and of nicotine (50 $\mu g.$) sufficient to paralyse the ganglia, as indicated by the absence of preliminary weakening of the heart. The immediate effect of the nicotine is toxic and decreased the response to acetylcholine but there was a slow recovery. A subsequent dose of nicotine again abolished the response to acetylcholine and to nicotine itself and again there was recovery.

are complex. A first injection of 0·005μg. nicotine causes inhibition, which is presumably due to stimulation of the parasympathetic ganglia, and this is followed by cardiac acceleration. When the ganglia are paralysed, subsequent injections of nicotine only stimulate the heart. At this stage, acetylcholine still slows the heart if no atropine is given, but stimulates it after atropine (Fig. 7). After further injections of nicotine, the heart muscle itself becomes poisoned, and the stimulating action of either nicotine, acetylcholine or adrenaline is abolished or very much reduced.

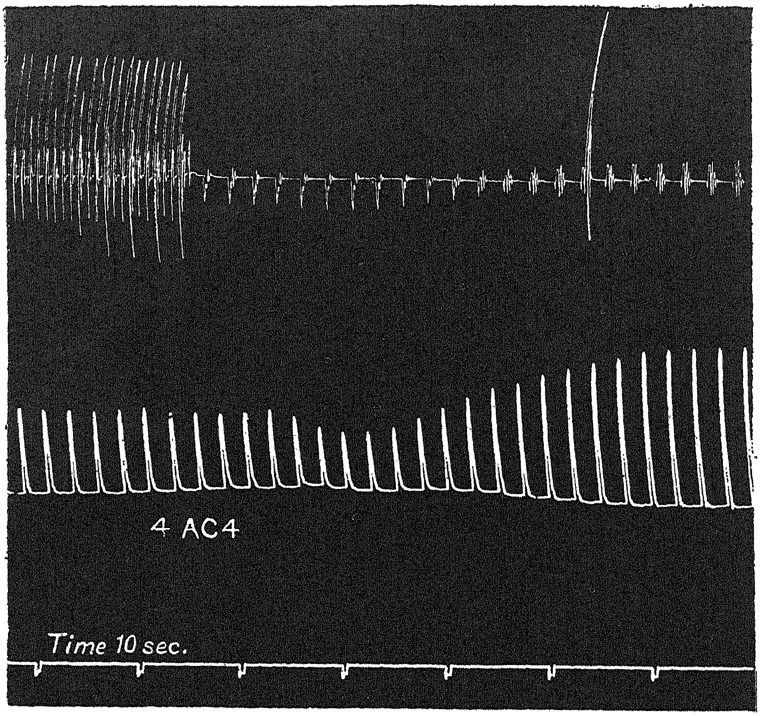

Fig. 8. Cat. The effects of acetylcholine (200μg.) in heart block produced by section of the A.V. bundle. When the auricle stopped beating the ventricular contractions continued to be transmitted slightly to the auricular lever. Upper record, auricle; lower record, ventricle.

Section of the auriculo-ventricular bundle greatly reduces the inhibiting action of acetylcholine on the ventricle but not on the auricle. In some hearts, as much as 500μg. acetylcholine have a negligible weakening effect only on the ventricle. This difference in the sensitivity of the auricle and ventricle to the depressant action of acetylcholine is evident from Figs. 8 and 9. The stimulating action of acetylcholine in suitable dosage, on the other hand, may affect the ventricle only. In the experiment shown in Fig. 8 heart block had been produced by section of the A.V. bundle; the injection of 200μg. acetylcholine

produced stoppage of the auricular contractions; the ventricular contractions, however, after an initial short period of slight depression, became much stronger. No atropine had been given in this experiment. The inhibiting action of acetylcholine on the ventricle is so small that it does not mask the stimulating effect. Before section of the bundle the effect of a similar dose of acetylcholine was strong inhibition of the ventricle. In the experiment of Fig. 9, section of the A.V. bundle had caused cessation of ventricular action; no atropine was given; the ventricle was caused to contract rhythmically by

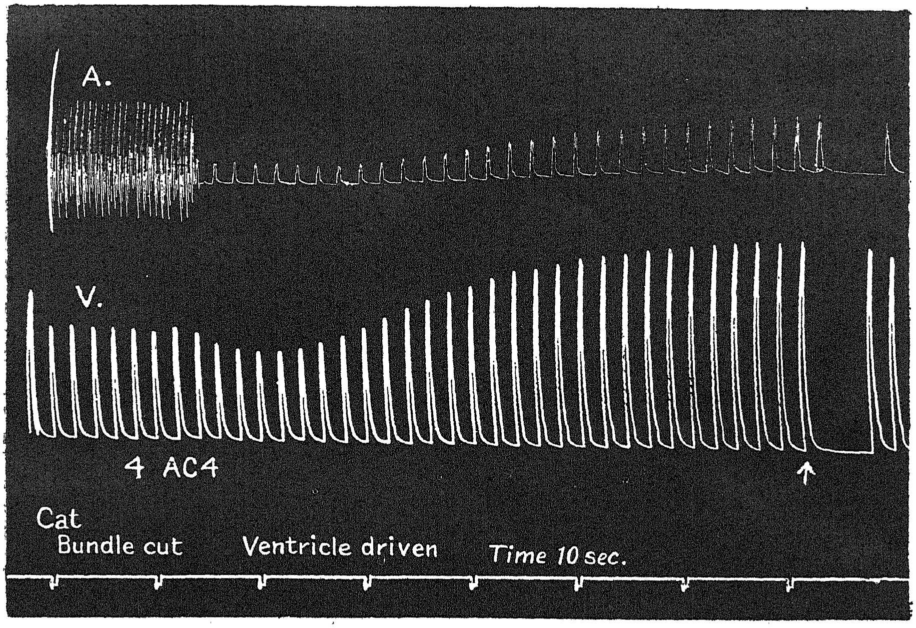

Fig. 9. Cat. The action of acetylcholine (200 μg.) immediately after section of the A.V. bundle, the heart being driven electrically. At the arrow the electrical drive was omitted for three stimulations. Upper tracing, auricle; lower tracing, ventricle.

electrical stimulation. The tracing again illustrates the difference in the action of acetylcholine on ventricular and auricular contractions. This difference may be seen even in hearts without section of the A.V. bundle. In weakly atropinized hearts acetylcholine sometimes still depresses the auricle but stimulates the ventricle (Fig. 10).

Comparison with adrenaline. When the stimulating action of acetylcholine on the atropinized heart is compared with that of adrenaline the following differences are noted: (1) Stimulation by acetylcholine in fresh hearts lasts longer than an equivalent stimulation by adrenaline. (2) On a heart not beating vigorously, adrenaline usually acts to increase the frequency and force,

acetylcholine more commonly affects the force only. Acceleration by acetylcholine may occur occasionally, but it is then more abrupt in onset than the acceleration produced by adrenaline. (3) Acetylcholine is more effective in equalizing irregularities of heart action, especially alternate beats of varying strength.

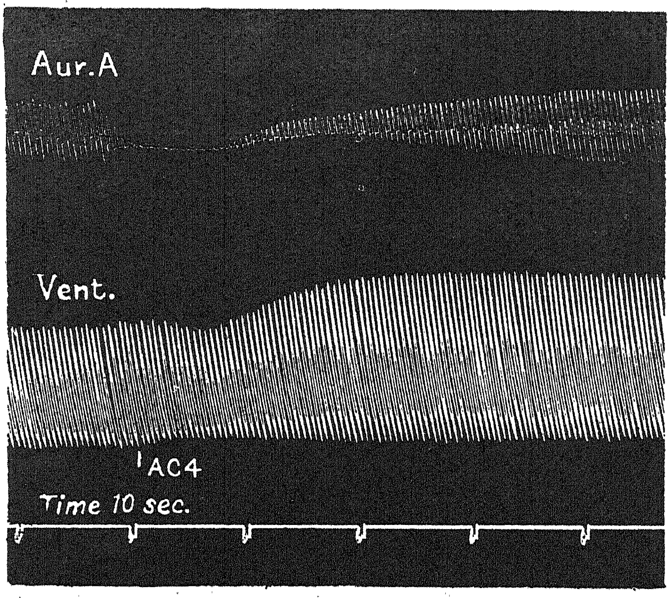

Fig. 10. Cat. The effect of acetylcholine (50μg.) on the auricle (upper tracing) and ventricle, showing the much greater sensitivity of the auricle after a dose of atropine (16·6μg.).

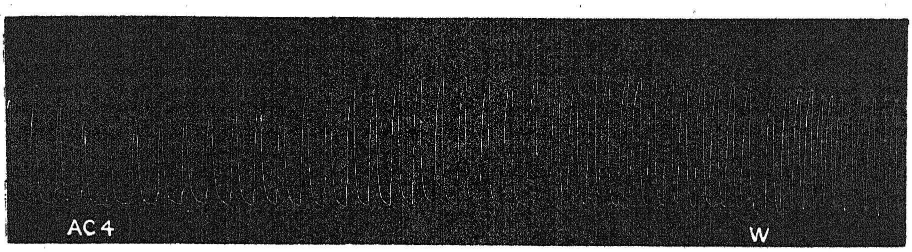

Fig. 11. The effect of acetylcholine (1 : 10,000) dropped on a suspended frog heart *in situ* and washed off at *W*. Note later acceleration.

Frog's heart. The stimulating effect of acetylcholine can also be demonstrated in experiments on the frog's heart. It is not, however, obtained so regularly as on the mammalian heart. If a drop of acetylcholine, 1 : 10,000, is applied to the frog's heart attached to a cardiograph in the usual way, slowing and weakening is seen. With smaller concentrations of acetylcholine

there is still slowing, but the heart beats vigorously (Fig. 11). If the acetylcholine solution has been warmed to counteract the slowing, the sole effect may be an increased force of contraction. The stimulating effect can be well demonstrated when the acetylcholine dropped on the heart is washed off a minute later. A heart which, before the acetylcholine treatment, was beating rather weakly may beat vigorously after washing out the acetylcholine. Controls with washing off drops of saline solution have no effect of this kind. Atropine, in doses just sufficient to abolish the inhibiting action, does not abolish the stimulating effect of acetylcholine, eserine enhances it.

DISCUSSION

The results given above may be looked upon as bridging a gap in our knowledge of the action of acetylcholine on the heart and on muscle. They show that, apart from the well-known action of acetylcholine in inhibiting impulse formation and conduction and in weakening auricular contraction, acetylcholine has a stimulating effect, especially on the ventricle. This effect is seen on the non-atropinized heart with minute doses of acetylcholine. Larger doses inhibit the ventricle, and, in order to unmask the stimulating effect of acetylcholine, atropine or methylene blue has to be given. When the A.V. bundle has been severed, however, large doses of acetylcholine may stimulate the ventricle even without atropine. It appears that the main action of acetylcholine on the ventricle is one of increasing the force of contraction.

The stimulating action of acetylcholine appears to be a direct one on the cardiac muscle, for it persists after the administration of nicotine in amounts apparently large enough to paralyse any ganglia which may be present.

Since acetylcholine is normally liberated by the vagus, the extent to which the stimulating action of the substance may be a physiological phenomenon requires consideration. In this connexion the fact that the effect is primarily one on the force of the heart may be considered of special importance, since vagal action on the heart under physiological conditions must always be associated with an increased force of contraction of the heart. It has already been pointed out (McDowall, 1926) that the development of vagus restraint of the heart is of special significance in relation to the output, and that its well-known development during physical training is presumably associated with greater efficiency of the cardiac muscle, which can then deal with the increased filling of a longer diastole. Hitherto, the increased force of contraction has been generally looked upon as purely a function of the cardiac muscle in accordance with Starling's Law of the Heart, and it has been presumed that vagal stimulation has no direct effect on the ventricular muscle, since the post-ganglionic vagal fibres do not innervate the ventricular muscle. If we assume, however, that some of the acetylcholine released by vagal action escapes to the ventricle, it would have a stimulating action on the force of the ventricular contraction.

It is, therefore, possible that the increased force of ventricular contraction during vagal slowing is in part a function of the cardiac muscle in accordance with Starling's Law of the Heart and in part an effect of the released acetylcholine.

What is probably more important physiologically is that when the inhibitory action of acetylcholine passes off, there follows a period during which there is an increased response to adrenaline. Thus it may be considered that when, in emotional stress or severe action, the normal vagus restraint of the heart is reduced, the heart is made all the more sensitive to sympathetic action. The value of such an arrangement is obvious, and how far it may be true that the parasympathetic in general acts as a sensitizer to the sympathetic is a field of further investigation. Such a possibility would explain the value of the apparently very extravagant continued release of acetylcholine by the parasympathetic in various parts of the body. This assumes that the acetylcholine released by the vagus has, like injected acetylcholine, access to the site of action of adrenaline; although this seems probable, it does not, however, exclude the possibility that the parasympathetic nerves may first liberate acetylcholine which subsequently acts on an adrenaline (or sympathin) releasing mechanism.

SUMMARY

1. It is shown that acetylcholine can, in certain circumstances, stimulate the heart. This occurs when it is administered in small doses to suitable hearts or at the commencement of the action of large doses or, with large doses, if the better known inhibiting action of the substance is abolished by atropine or methylene blue.

2. The stimulating action is enhanced by eserine after atropine, but may be abolished by ergotoxine or large doses of atropine.

3. The stimulating action is seen after the administration of nicotine in sufficient dosage to paralyse autonomic ganglia and if the ventricular muscle is driven electrically. It therefore appears to be a direct action on the cardiac muscle.

4. The effect, compared with that of adrenaline, is more on the force of the heart than on the frequency. The possible significance of these facts in the intact animal is discussed.

In conclusion, I should like to thank Dr D. B. Taylor for confirming many of the results in his classes in Pharmacology, and also Mrs Lynn, Miss Lee and Mr Stonard, whose excellent performance of routine duties for two years in a laboratory peculiarly exposed to enemy action, contributed greatly to the success of the work.

Note added in Proof. Since this paper was submitted, a paper by Hoffmann, Middleton & Talesnik (*Amer. J. Physiol* (1945), **144**, 189) has appeared confirming the main results and adding the observation that the stimulating

action of acetylcholine is abolished by curare and is accompanied by a release of adrenaline into the coronary outflow. The results on which my paper is based had been communicated to the Physiological Society in June and November 1944.

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NORMAL VARIATIONS IN BLOOD HAEMOGLOBIN CONCENTRATION

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In attempting to assess the significance of changes in haemoglobin levels following burns (Brown, 1944), it was found necessary to reinvestigate the variations which occur in normal individuals. Dreyer, Bazett & Pierce (1920) recorded the haemoglobin levels of normal adults at intervals during the day, and found a maximum variation of 30 %. They regarded changes of 10 % as common. Rabinovitch (1923) noted a diurnal variation of 26 % in two cases, 15–20 % in four, and 10–15 % in six. Short (1935) reported a possible variation of 17 %. Considerably less variation was found by McCarthy & Van Slyke (1939) and McCarthy (1943). In eighteen cases the average range between the highest and the lowest values was 1·3 vol. %. The greatest observed range was 2·3 vol. %, which was equivalent to 11 % of the mean haemoglobin for the day.

It is evident from this published work that haemoglobin levels vary significantly during the day, but there is little agreement on the magnitude of the variation which is to be regarded as normal.

METHODS

The subjects chosen for the investigation comprised (*a*) healthy senior medical students and resident medical officers, who were pursuing their usual routine during the period of observation, and (*b*) a series of adults confined to bed. In the latter group the patients were either awaiting operation for an anatomical defect such as hernia, or suffering from some minor complaint not associated with any disturbance of fluid balance (Tables 1 and 2). Investigations on the day-to-day variations in haemoglobin were made on both groups, and samples of venous blood were withdrawn between 11.00 and 11.15 a.m. daily for 4–5 days. Investigations on diurnal variations in haemoglobin were made only on the patients confined to bed. Samples of venous blood were obtained at intervals of 3 hr. from 9 a.m. until 6 p.m.

Samples of venous blood were collected through a dry sterile needle into a test-tube containing a sufficiency of dry heparin. Temporary stasis was induced in order to facilitate bleeding. The stasis was of short duration (less than 90 sec.) and would not influence haemoglobin levels (Brown, 1945). Haemoglobin and haematocrit estimations were made on all samples. The results were not significantly different, and only the haemoglobin values are recorded here.

Haemoglobin was estimated in duplicate, with oxyhaemoglobin as the pigment, in a double-cell mains-operated photoelectric colorimeter designed by one of us (A.B.). The instrument incorporates a modification of the compensating circuit described by Brice (1937). It was calibrated

against oxygen capacity using Hüfner's factor. The filters employed were Chance blue-green and Wratten No. 88. The maximum sensitivity of the instrument is equivalent to 0·03 g. Hb/100 ml. In practice the results in duplicate agreed to within 0·15 g. The stability of the instrument was checked throughout the work by the periodic insertion of a Wratten neutral screen. No change greater than that equivalent to 0·03 g. was observed during the period of study. The solution of oxyhaemoglobin was prepared for estimation by adding 0·100 ml. of blood to 10 ml. of 0·4 % ammonia. Grade 'A' (N.P.L.) pipettes were used throughout the work.

The haematocrit estimation was carried out by the method of Wintrobe (1933).

RESULTS

The results of the examination of the blood of twenty-four individuals at the same time daily for 4–5 days are shown in Table 1. The maximum variation recorded in any case over the entire period of the investigation was 2·05 g./100 ml., equivalent to 14·7 % of the mean haemoglobin for the period. The average maximum variation was about half the greatest recorded, and equal to 6·5 % of the average mean haemoglobin. The variation in any period of 24 hr. was less marked. It is to be noted that only one patient showed a progressive change to account for this (Case 3).

Table 1. Variations in haemoglobin

Case no.	Daily haemoglobin values (g./100 ml.) 1	2	3	4	5	Remarks
1	13·00	13·30	13·65	12·95	—	Healthy adult male
2	14·85	14·65	14·25	14·80	—	Healthy adult male
3	14·35	13·45	13·70	12·65	—	Healthy woman: menstruating
4	17·10	16·50	15·65	16·25	—	Healthy adult male
5	14·80	14·25	14·55	15·00	14·60	Healthy adult male
6	13·90	13·20	13·50	14·00	13·80	Healthy adult male
7	13·35	12·90	12·85	12·80	13·30	Healthy adult male
8	15·05	14·30	14·80	15·00	—	Healthy adult male
9	13·75	14·35	14·30	14·70	14·10	Healthy adult male
10	15·10	15·15	14·85	15·30	14·85	Healthy adult male
11	14·80	14·95	14·70	14·80	14·40	Healthy adult male
12	15·05	13·65	13·40	13·00	14·15	Healthy adult male
13	12·05	11·10	12·10	12·35	12·70	Dyspepsia
14	11·40	12·10	12·15	12·00	11·80	Chronic arthritis
15	15·15	15·45	15·25	15·20	—	Hyperacidity
16	14·50	15·35	15·00	15·45	15·10	Hyperacidity
17	14·75	14·50	14·60	14·95	14·05	Lipoma
18	15·65	15·50	15·50	14·90	—	Hernia
19	15·70	16·05	15·65	15·40	15·15	Dyspepsia
20	15·65	15·35	15·45	15·95	15·35	Old injury
21	15·35	15·30	15·60	15·70	15·70	Arthritis
22	15·45	15·80	16·95	15·90	15·50	Hyperacidity
23	14·60	14·40	14·70	15·15	14·15	Dyspepsia
24	14·65	15·25	15·65	16·10	14·95	Hernia

The ten individuals in whom diurnal changes were investigated formed a separate series, and the results and relevant details are shown in Table 2. The maximum variation was 1·0 g./100 ml., equivalent to 6·9 % of the mean: the average maximum was 4·12 % of the mean. No uniform direction of change was encountered, and it was impossible to predict a period during which the greatest change might be expected to occur.

TABLE 2. Diurnal variations in haemoglobin

Case no.	3-hourly haemoglobin values (g./100 ml.) 9 a.m.	12 noon	3 p.m.	6 p.m.	Remarks
1	13·60	13·40	13·50	13·45	Peptic ulcer
2	14·10	14·20	15·10	14·50	Peptic ulcer
3	12·30	12·20	11·80	12·40	Anal stricture
4	12·95	13·30	12·75	13·40	Hernia
5	13·20	13·40	13·40	13·90	Cyst of toe
6	12·70	13·50	13·00	12·90	Hernia
7	10·83	11·30	10·95	11·20	Peptic ulcer
8	12·30	12·55	12·55	12·60	Painful scar
9	12·90	12·55	12·55	12·85	Old injury
10	13·50	13·40	13·70	13·50	Chronic cholecystitis

From consideration of the results in both series it is evident that the more frequently estimations were made in a given period of time, the greater was the variation which was revealed. The average maximum change in haemoglobin levels at intervals of 9 hr. was 2·07 % of the mean, while the average maximum change recorded in 3-hourly estimations over the same period was 4·12 % of the mean. When four estimations were made over a period of 72 hr. the average maximum change was 5·67 % of the mean. Thus both the number of estimations and also the length of the period over which they are made may influence the magnitude of the variations recorded—at least within a 4–5 day period.

DISCUSSION

These results, obtained by the careful use of accurate photoelectric methods, are in close agreement with the diurnal changes recorded by McCarthy & Van Slyke (1939). The greater diurnal variations recorded by Dreyer *et al.* (1920) have not been confirmed: the observations in this instance were, however, made on capillary blood. The method used by Rabinovitch (1923) has been regarded as unsatisfactory (McCarthy & Van Slyke, 1939), and no duplicate estimations are recorded. Short (1935) reported a maximum variation of 17 % based on red-cell counts and haemoglobin estimations which in duplicate differed by as much as 540,000 per cu.mm. and 0·8 g. % respectively. The method of estimation of haemoglobin is not mentioned.

McCarthy & Van Slyke have criticized the colorimetric estimation of haemoglobin on the grounds that variable factors in the plasma might alter light absorption and so introduce errors. Even if they were to do so, the apparent diurnal variation would be increased in comparison with the results of these authors, not diminished as in the present investigation.

Variations in the distribution of the red cells throughout the vascular system seem the most probable cause of the fluctuations in haemoglobin concentration described in this report. The reservoir function of the spleen may be important in this respect. There is no evidence that oral fluid intake is in any way responsible. Observations on the effect of rapid ingestion of 400 ml. of water in eight healthy subjects have failed to reveal any effect on

haemoglobin concentration. On the other hand, splenic contraction may result from exercise or excitement, and haemoconcentration may accompany such a change. In the present study, splenic contraction may have resulted from excitement in anticipation of venepuncture. Other sources of red cells may participate. Variations in haemoglobin such as are recorded here are therefore to be reviewed in relation to circumstances. They must be regarded as normal in any investigation in which repeated venepuncture is performed. They may, however, be in excess of the changes which occur in a normal person who is physically and mentally at rest.

SUMMARY

In ten patients without evidence of active disease and confined to bed, the average maximum diurnal variation in haemoglobin (from values obtained from venous blood at intervals of 3 hr. between 9 a.m. and 6 p.m.) was 0·54 g./100 ml., equivalent to 4·12 % of the mean haemoglobin for the period. The greatest diurnal variation recorded was 1 g. or 6·9 % of the mean.

Daily estimation of haemoglobin in twenty-four adults, of whom eleven were ambulant and thirteen confined to bed, over a period of 4–5 days revealed an average maximum variation of 0·93 g./100 ml., or 6·3 % of the mean value for the period. The greatest variation recorded in the period was 2·05 g./100 ml., or 14·7 % of the mean.

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THE EFFECTS OF ADRENALINE ON BLOOD HAEMOGLOBIN CONCENTRATION

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Investigations on the changes in the blood and blood pressure in relation to burns (Brown, 1944) have shown that even with minor injuries the blood pressure may be raised and slight haemoconcentration may occur. These findings may be related, and both may be due to excitement. Adrenaline plays a prominent part in the production of the excitement syndrome, and it was thought that, by observing the effect of the drug on haemoglobin levels, some idea would be gained as to the possible magnitude of the resulting changes in man caused by excitement.

METHODS

In the course of investigations on the glycogenolytic action of adrenaline in cases of hepatic disease, advantage was taken of the opportunity to observe the effect of the drug on haemoglobin levels. The nature of the primary investigation explains the relative frequency of liver disease among the patients selected. Several relatively normal individuals have also been included.

The patients were confined to bed on the day of the test which began at about 11.15 a.m. and ended before the midday meal. Samples of blood were obtained 5 min. before, and 10, 30 and 60 min. after, the subcutaneous injection of 1 ml. of 0·1 % adrenaline hydrochloride. Two patients were given 1 ml. of 0·85 % sodium chloride instead of adrenaline.

Venous stasis of short duration (less than 60 sec.) was used to facilitate venepuncture. Such stasis does not influence haemoglobin levels (Brown, 1945). Blood was collected in a clean dry tube containing a suitable quantity of heparin as an anticoagulant.

Haemoglobin was estimated in a Spekker photoelectric colorimeter with oxyhaemoglobin as the pigment, 0·100 ml. of blood being added to 10 ml. of 0·4 % ammonia. The pipettes used were Grade 'A' (N.P.L.). All estimations were performed in duplicate, and no two such estimations differed by more than 0·15 g. %.

RESULTS

The changes in haemoglobin levels in sixteen patients given 1 mg. of adrenaline hydrochloride are shown in Table 1. With three exceptions, a significant rise in haemoglobin followed the injection. It reached a maximum in 10 min. when an average rise of 0·73 g., equivalent to 5·53 % of the mean original level, was recorded. A fall occurred in 30 min., and return to pre-adrenaline levels was noted at the end of 1 hr. There is no evidence that the results were affected by the nature of the illness present.

TABLE 1. The effect of adrenaline on haemoglobin concentration

Case no.	1st Hb	Time after adrenaline 10 min.	30 min.	60 min.	Max. rise	Max. % of 1st	Remarks
1	11·30	11·95	11·65	11·50	0·65	5·75	Hepatic cirrhosis
2	9·95	10·50	10·70	9·95	0·75	7·54	Hepatic cirrhosis
3	12·90	13·60	13·40	12·90	0·70	5·42	Healed peptic ulcer
4	13·05	13·55	13·40	13·05	0·50	3·83	Healed peptic ulcer
5	12·95	13·65	13·00	13·00	0·70	5·38	Healed peptic ulcer
6	12·90	13·40	13·10	12·70	0·50	3·94	Sciatica
7	12·65	12·95	12·60	12·70	0·30	2·36	Infective hepatitis
8	15·05	15·35	14·95	15·00	0·30	2·00	Healed pneumonia
9	15·30	16·45	15·55	15·25	1·25	8·20	Fibrositis
10	14·90	15·55	15·30	14·90	0·65	4·36	Intestinal taeniasis
11	11·30	11·25	10·20	9·90	Fall	—	Fibrositis
12	11·70	11·00	11·55	10·80	Fall	—	Healthy male
13	12·25	12·80	12·20	11·85	0·55	4·64	Infective hepatitis
14	13·85	15·40	15·60	15·30	1·55	10·01	Infective hepatitis
15	13·65	14·70	14·60	13·75	1·15	8·48	Infective hepatitis
16	11·20	11·05	10·70	10·70	Fall	—	? Lymphadenoma
17	10·70	10·45	10·70	10·85	Fall	—	No adrenaline given
18	14·80	14·60	14·45	14·15	Fall	—	No adrenaline given
Average (excluding 11, 12, 16–18)					0·73	5·53	

Hb levels in the table are recorded in g./100 ml. of blood.

DISCUSSION

The haemoconcentration observed in this investigation is probably due to the liberation of red cells in a concentrated form from the spleen. Since the original work of Barcroft and his colleagues (1922), it has become increasingly evident that the spleen, in animals at least, acts as a blood reservoir which adjusts the circulating haemoglobin according to the requirements of the moment. Conditions associated with anoxaemia (Barcroft, Harris, Orahovats & Weiss, 1925; Barcroft & Stephens, 1927), and excitement (Hargis & Mann, 1925; Izquierdo & Cannon, 1928) cause splenic contraction, and peripheral haemoconcentration may follow. In man also the spleen may have a similar function. Injection of adrenaline into the splenic artery before splenectomy for various conditions was found to cause contraction of the spleen and haemoconcentration in the splenic vein (Watson & Paine, 1943), and, contrary to the findings in the intact individual, splenectomized patients showed no significant alteration in the red-cell levels of the peripheral blood following the administration of adrenaline (Benhamou, Jude & Marchioni, 1929).

The maximum rise in haemoglobin of 11·2 % observed in the present study falls short of the 20 % rise caused by excitement in animals (Izquierdo & Cannon, 1928), and by adrenaline in man (Benhamou *et al.* 1929). Nevertheless, the rise is sufficiently great to indicate that in man a sympathomimetic drug may cause significant haemoconcentration. The investigation does not prove that excitement would have a similar effect, but the possibility cannot be doubted. Indeed, the unusual response noted in cases 11, 12 and 16, and the changes in those given only saline, may be explained on this basis alone.

SUMMARY

The changes in the haemoglobin concentration in the venous blood of sixteen patients given 1 mg. of adrenaline hydrochloride subcutaneously have been described. With three exceptions, a significant rise in haemoglobin followed the injection. The highest levels were recorded after 10 min. when an average rise of 0·73 g./100 ml., equivalent to 5·5 % of the mean original level, was recorded. The maximum rise was 1·55 g./100 ml., equivalent to 11·2 % of the original level.

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PROPERTIES AND DISTRIBUTION OF THE ENZYME SYSTEM WHICH SYNTHESIZES ACETYLCHOLINE IN NERVOUS TISSUE

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Both homogenized brain tissue and cell-free extracts prepared from acetone-dried brain contain an enzyme system which synthesizes acetylcholine, provided that adenosinetriphosphate (ATP) is added (Nachmansohn & Machado, 1943; Nachmansohn, John & Waelsch, 1943; Feldberg & Mann, 1944, 1945*a*; Nachmansohn & John, 1944, 1945). We have shown previously (1944, 1945*a*) that cell-free solutions prepared by extraction of 1 g. acetone-dried and powdered brain can synthesize anaerobically between 140 and 400 μg. acetylcholine/hr. at 37° C. Only about half this amount of acetylcholine was formed aerobically, but reduced glutathione and cysteine brought the aerobic synthesis to almost the same level as that observed anaerobically. Glucose inhibited the formation of acetylcholine owing to the esterification of the labile phosphate groups of ATP.

Recently it has been found (Feldberg & Mann, 1945*b*) that ATP is not the only factor involved in the formation of acetylcholine in cell-free extracts. The brain itself contains a substance which can be extracted with boiling water or 0·9% NaCl solution from the fresh or acetone-dried tissue. This substance is not identical with ATP, yet greatly increases the formation of acetylcholine. We shall refer to it throughout this paper as the 'activator'. Moreover, ATP and the activator are not the only two thermostable factors which influence the formation of acetylcholine. Nachmansohn & John (1944, 1945) have found that enzyme preparations from brain which have been inactivated by dialysis could be restored to their full activity by the addition of *l*-(+)-glutamic acid and citric acid. We have been able to confirm the effect of citric acid; at the same time, however, we have noticed that citric acid does not merely restore the enzyme activity of dialysed brain extracts, but also greatly increases the formation of acetylcholine in non-dialysed extracts. We have found that the activator and citrate together can increase the synthesis of acetylcholine as much as or even more than ATP alone. This shows that

ATP is not an irreplaceable component of the synthesizing system. Yet, if ATP is included in the system which already possesses the activator and citrate, then the rate of synthesis is further increased, so that as much as 1800 μg. acetylcholine can be formed/g. brain powder/hr., i.e. 6 mg. acetylcholine/g. dry material contained in the synthesizing extract from brain. The high rate of acetylcholine formation under such conditions thus provides a very sensitive method for investigating the capacity of nervous tissue to synthesize acetylcholine and has been used in this way to compare, among other things, the synthesizing power of motor and sensory spinal roots.

METHODS

The experimental methods were essentially the same as those previously described (Feldberg & Mann, 1945*a*). Certain abbreviated descriptions are needed in this paper and are to be read with the following meaning:

Brain. This refers to brain tissue anterior to a section across the brain stem between the anterior and posterior corpora quadrigemina. It includes therefore the cerebral hemispheres, basal ganglia and the part of the stem anterior to the section.

Brain stem. This refers to the same portion of the brain as above, but from which the cerebral hemispheres have been removed. It includes therefore the basal ganglia and the part of the stem anterior to the section.

The enzyme system which synthesizes acetylcholine was obtained either from brain or from peripheral nerves. The tissue was dried with acetone and ground with saline solution (0·9 % NaCl). 1 g. acetone-dried brain powder corresponds to about 5·5 g. fresh brain tissue in the case of the rat, guinea-pig and rabbit, to about 4·8 g. in the dog and cat and to about 9 g. in a 1-day-old kitten; 1 g. acetone-dried tissue of the peripheral nerves corresponds to about 4 g. fresh tissue. The saline extracts from acetone-dried brain were centrifuged before use; they contained about 30 % of the dry material originally present in the acetone powder. In the case of the peripheral nerve tissue the centrifugation was omitted. The incubated samples had a volume of 4·7–5 c.c. and included the following: saline extract from 50 mg. acetone-dried tissue, 6 mg. KCl, 3 mg. choline, 2 mg. NaF, 0·5 mg. eserine sulphate, 1·5 mg. $MgSO_4$, 0·4 mg. ATP-pyro-P and 4·5 mg. cysteine. The samples were buffered with sodium phosphate, pH 7, and incubated for 1 hr. at 37° C. It should be pointed out that, although eserine was included in the incubated samples, this was done merely as a precaution in case some cholinesterase might have been present. As a rule, however, extracts from acetone-dried brain contain very little or no cholinesterase.

The dialysis of saline extracts from brain was carried out in cellophan tubes against saline solution for 15–20 hr. at 0–2° C. The dialysed extracts were opalescent, but the turbidity could be removed without impairing the enzyme activity by treating the extracts with a small quantity of tricalcium phosphate gel and centrifuging.

The activator was usually obtained from acetone-dried brain by mixing 1 part acetone powder with 10–15 parts of saline solution and boiling the mixture for 2–3 min. The boiled mixture was filtered through cotton-wool or centrifuged.

RESULTS

The 'Activator'

Table 1 shows the amounts of acetylcholine synthesized by non-dialysed saline extracts from acetone-dried brain. In the presence of ATP, the extracts prepared from rat brain synthesized anaerobically in 1 hr. at 37° C. from 280 to 430 μg. acetylcholine/g. acetone powder. Under similar conditions the

TABLE 1. Formation of acetylcholine in saline extracts from acetone-dried brain. Effect of ATP, activator and citrate

Animal	μg. acetylcholine synthesized in 1 hr./g. acetone powder in the presence of ATP	ATP and activator	ATP and citrate	ATP, activator and citrate
Rat	280	680	1070	1200
	290	—	1050	—
	290	740	1000	—
	300	—	950	—
	300	600	1000	1100
	320	—	850	—
	320	600	1000	—
	320	—	1000	—
	320	750	1070	—
	350	—	960	—
	430	1000	1160	—
Guinea-pig	320	—	1000	—
	640	800	1010	1450
	740	950	1150	1800
			1240	—
Rabbit	260	—	720	1280
			600	1050
Cat	130	—	480	580
Kitten (1 day old)	60	—	180	140
Dog	35	105	320	400
	60	140	300	360
	90	—	380	480

amounts of acetylcholine formed by extracts from brain of other animals were as follows: guinea-pig 320–740; rabbit 260; cat 130; 1-day-old kitten 60; dog 35–90 μg./g. The activator, added in the form of the boiled juice, brought the synthesis of acetylcholine to the following levels: rat 600–1000; guinea-pig 800–950; dog 105–140 μg./g. In these experiments each incubated sample contained the saline extract from 50 mg. and added activator from 100 mg. acetone powder. Such an amount of activator was found to have the optimal effect, and it made little difference whether it had been obtained from acetone-dried or fresh brain. There was, moreover, little difference between activator preparations obtained from different species of animals.

The above experiments were carried out under anaerobic conditions. Aerobically the effect of activator on the synthesis of acetylcholine was very much the same as under anaerobic conditions, but the aerobic synthesis of acetylcholine was frequently about 10–20% lower than the anaerobic synthesis.

The most frequently applied method for the preparation of the activator was to grind the acetone-dried brain in saline solution, to boil the mixture and to filter it afterwards. However, the same activity was obtained if the mixture of acetone powder and saline solution was first centrifuged and only the supernatant fluid boiled. This point deserves special mention because it

shows that the unboiled centrifuged saline extract from acetone-dried brain contains both the enzyme which synthesizes acetylcholine and the activator. In other words, if boiled juice obtained from 100 mg. acetone powder is mixed with the unboiled saline extract, prepared from 50 mg. acetone powder, then the total amount of the activator present in the mixture corresponds to that of 150 mg. powder. If, on the other hand, the sample contains only the unboiled saline extract from 50 mg. acetone powder without additional boiled juice, then the activator only corresponds to 50 mg. powder.

The yield of acetylcholine depends not so much on the absolute amount of the activator present but rather on its relative concentration in the incubated sample. This was shown in the following experiment. Three samples were set up with volumes of 3·7, 4·7 and 6 c.c., respectively. They all contained the same amount of unboiled brain extract corresponding to 50 mg. brain powder, and yet the yield of acetylcholine in the three samples was not the same. The first sample synthesized 380 μg./g., whereas the other two produced 280 and 210 μg./g. respectively. However, if boiled juice was added to the three samples in such amounts as to bring the concentration of the activator to approximately the same level, then they all produced the same amount of acetylcholine. The first sample was treated with boiled juice from 50 mg. acetone powder, and it produced 640 μg./g. acetylcholine. The second and third samples received the boiled juice from 100 and 175 mg. powder, respectively, and they yielded 700 and 620 μg./g. acetylcholine.

The activator is not a mineral constituent; in fact, the addition of ash from boiled juice caused an inhibition of the synthesis of acetylcholine.

The activator is not identical with either choline, KCl or ATP. This was shown by the following experiments. First, the effect of boiled juice was compared with that of choline and KCl. It was found that, whereas the synthesis of acetylcholine became higher with increasing amounts of boiled juice, it was not changed by increasing the amount of choline up to 9 mg. choline per sample; similarly, an increase of KCl from 6 to 18 mg. per sample increased the yield of acetylcholine by not more than 20 %. That the activator was not identical with ATP was shown as follows:

(1) It was found that in the presence of ATP the rate of acetylcholine formation was increased by the addition of boiled juice, whereas any further increase in ATP had no effect.

(2) The boiled juice was analysed for its content of ATP. This was done by a procedure based on the method of Parnas & Lutwak-Mann (1935) for the estimation of ATP in muscle. Only 0·03 mg. ATP-labile P was found in 2 g. acetone powder. The amount of boiled juice usually added to incubated samples corresponded to 100 mg. acetone powder and represented therefore only 0·0015 mg. ATP-P. This means that by the addition of boiled juice the ATP in the samples was increased by not more than 1/250.

(3) The stability of the activator differs markedly from that of ATP, and preparations of the activator are slowly inactivated even by standing at 0° C. This is shown by the following observations. When the activator was prepared by boiling a saline extract from acetone-dried brain, it was noticed that much more active preparations were obtained from fresh saline extract than from an extract which had been standing for 24 hr. at 0° C. before being boiled. This inactivation might be due to an enzymic decomposition of the activator by an enzyme present in the unboiled saline extract, but this is not so, since an equally large inactivation occurred when the saline extract from brain was boiled immediately after it had been prepared and then the boiled juice left standing for 24 hr. at 0° C. In an experiment in which the saline extract had been obtained from acetone-dried rat's brain it was found that in 1 hr. at 37° C., the amounts of acetylcholine synthesized were as follows: with the saline extract alone 320 μg./g.; on addition of boiled juice, prepared from the fresh saline extract and used immediately after boiling, 740 μg./g.; on addition of boiled juice prepared from a fresh saline extract, but used after the boiled juice had been standing at 0° C. for 24 hr., 560 μg./g.; with boiled juice, prepared from a saline extract which had been standing at 0° C. for 24 hr. before boiling, 560 μg./g.

The activator is a dialysable substance; a boiled juice used after dialysis for 24 hr. had not more than 10–20% of its original activity. Since the activator is also present in the unboiled saline extract prepared from acetone-dried brain one would expect that such an extract, if dialysed, would lose the activator without losing the enzyme which synthesizes acetylcholine. Indeed, a saline extract from acetone-dried brain powder which has been dialysed for 16–24 hr. is hardly capable of synthesizing acetylcholine even if ATP is added to it. However, if ATP and a freshly prepared boiled juice are added to the dialysed extract together, then large amounts of acetylcholine are formed both under anaerobic as well as aerobic conditions (Table 2).

TABLE 2. Formation of acetylcholine in dialysed saline extracts from acetone-dried brain in the presence and in the absence of ATP

μg. acetylcholine synthesized in 1 hr. in dialysed extract of 1 g. acetone powder

	Additions				
Animal	None	Activator	Citrate	Activator and citrate	Notes
Guinea-pig	20	470	900	1100	Samples contained ATP
Rat	50	500	680	1100	Samples contained ATP
Rat	0	40	30	310	Samples contained no ATP

Investigations were made to determine whether the activator is a substance present specifically in the brain or if it occurs in other tissues. It was found that both the yeast 'Kochsaft' as well as the boiled juice prepared from skeletal muscles have some activating effect on the synthesis in dialysed saline

extracts from brain. These effects, however, were much smaller than the effect of boiled brain juice. On the other hand, a fairly active preparation of the activator was obtained from the liver. This is shown by the following experiment. A saline extract was prepared from acetone-dried brain of guinea-pig and was then dialysed for 20 hr. The dialysed extract plus ATP synthesized 16 μg. acetylcholine/g. acetone powder/hr. On the addition of boiled juice preparations obtained from brain, liver, muscle and yeast the levels of acetylcholine rose to 480, 470, 100 and 125 μg./g. respectively.

Purified preparations of cozymase and of reduced glutathione could not replace the activator.

Citric acid

Citric acid greatly increases the synthesis of acetylcholine in samples incubated with ATP and saline extracts from acetone-dried brain. Table 1 shows the effect of 0·02 M-sodium citrate on the formation of acetylcholine in extracts prepared from brain of various animals. But even with much smaller concentrations the effect of citrate is well pronounced. The optimal concentration is about 0·004 M., and some activation is noticeable with as little as 0·000004 M-citrate (Table 3). The action of citrate appears to be independent

TABLE 3. Effect of different concentrations of sodium citrate on the aerobic formation of acetylcholine in saline extracts from acetone-dried rat's brain in the presence and in the absence of ATP

Molar concentration of sodium citrate added to samples	μg. acetylcholine formed in 1 hr./g. acetone powder: With ATP	Without ATP but with activator
0	300	50
0·000004	380	55
0·000016	450	40
0·00008	660	65
0·00025	720	110
0·0008	900	340
0·0033	950	370
0·01	950	—

of the presence of oxygen. In fact, in some experiments, a slightly higher level of synthesis was observed in samples incubated with citrate under aerobic than anaerobic conditions, in spite of the fact that without citrate the formation of acetylcholine is frequently about 10–20% lower under aerobic than anaerobic conditions.

As previously shown (Feldberg & Mann, 1945*a*), the aerobic synthesis is reduced by about 50% if cysteine is not included in the incubation mixture. However, the aerobic formation of acetylcholine in samples in which citrate had been added was only about 10% smaller in the absence of cysteine.

In Table 1 the effect of citrate is compared with that of the activator. In non-dialysed brain extracts the level of acetylcholine formation is brought to an even higher level by the addition of citrate than by the addition of

boiled juice. If both citrate and the activator are added together, then the level is increased still further. Similar behaviour is shown by the addition of citrate and the activator to dialysed brain extracts, both under aerobic and anaerobic conditions (Table 2).

Citric acid might act by removing the calcium ions which are known to inhibit the enzymic formation of acetylcholine. The dialysed saline extract obtained from 1 g. acetone-dried rat's brain was found by analysis to contain 0·17 mg. Ca. Considering the small amount of the extract which was added to incubated samples it appears unlikely that the calcium present in the extract (1·7 μg./c.c. in the sample) could have had any appreciable effect on

TABLE 4. Effect of different acids on the formation of acetylcholine in saline extracts from acetone-dried brain in the presence of ATP

Acid	μg. acetylcholine formed in 1 hr./g. acetone powder: Without acid	With 0·01–0·02 M-acid	Notes
d, l-glutamic	300	300	
	320	480	
	430	460	
	15	45	Dialysed saline extracts
l-(+)-glutamic	210	220	
	210	230	
	210	390	
	210	250	
	220	280	
	240	320	
	240	680	
	10	32	Dialysed saline extracts
	15	140	Dialysed saline extracts
Aconitic	280	420	
	300	400	
	640	360	Activator added to samples
	680	540	Activator added to samples
Succinic	320	340	
	320	160	
Fumaric	320	230	
Acetic	340	290	
	620	740	Activator added to samples
Oxalic	290	220	
	320	300	
Tartaric	290	290	
Pyrophosphoric	290	200	
Malonic	320	440	
	300	240	
	900	930	Samples contained citrate
	1000	1040	Samples contained citrate
	1450	1600	Samples contained citrate
Pyruvic	350	65	
	320	15	
	320	70	
Oxaloacetic	220	14	
	240	40	

the synthesis of acetylcholine. This was corroborated by the finding that the addition of sodium oxalate had no effect on the synthesis of acetylcholine, although oxalate is also a Ca-binding substance.

In reactions associated with the so-called 'citric acid cycle' of the liver, malonic acid acts as an inhibitor. The addition of malonic acid was not, however, found to cancel the effect of citric acid on the formation of acetylcholine in the presence of ATP (Table 4).

Other organic acids

In view of the great activating influence of citric acid, other organic acids, especially those which are related to citric acid, might also be thought to exert some influence on the formation of acetylcholine. Other acids examined are listed in Table 4. No acid had an accelerating effect in any way comparable with that of citric acid. Inconsistent results were obtained with glutamic acid in experiments with both non-dialysed and dialysed brain extracts. Aconitic acid had some stimulating action. We thought that this small effect might be enhanced by the addition of the boiled juice from brain, but found that more acetylcholine was produced in the presence of boiled juice alone than if both aconitic acid and the boiled juice were added together.

The following acids were without effect or produced a slight inhibition: succinic, fumaric, oxalic, acetic, tartaric and pyrophosphoric acids. The small inhibition caused by some of these acids was not on a scale comparable with that of pyruvic and oxaloacetic acid. It has already been shown by Nachmansohn & John (1944, 1945) that keto-acids act as strong inhibitors of the formation of acetylcholine.

Comparison between the action of ATP, activator and citric acid

In the experiments so far described the synthesis of acetylcholine was always studied in the presence of ATP. However, when comparing the action of the three factors, ATP, the activator and citric acid, it was found that none of the three factors, if present alone, can promote to any appreciable extent the synthesis of acetylcholine in the isolated enzyme system, and that ATP is not an irreplaceable component of the synthesizing system, for a large synthesis occurs if the activator and citrate are added to the enzyme, in spite of the absence of ATP.

In comparing the three factors, ATP, the activator and citric acid, the experiments with non-dialysed and dialysed brain extracts have to be considered separately. If ATP alone or citrate alone are added to the non-dialysed extract prepared from acetone-dried rat's brain, then the level of acetylcholine synthesis rises from 8 to 360 μg./g. and to 230 μg./g. respectively (Table 5). However, it should be realized that these effects are not due solely to ATP or citrate alone, but must be attributed to a combined action of

TABLE 5. Formation of acetylcholine in saline extracts from acetone-dried brains of different animals in the presence and absence of ATP

μg. acetylcholine formed in 1 hr./g. acetone powder

	Additions					
Animal	None	Activator	Citrate	Activator and citrate	ATP	ATP and citrate
Rat	8	32	230	410	360	1110
Guinea-pig	12	17	105	220	530	1080
Rabbit	—	—	—	190	220	720
Cat	—	—	—	190	130	480
Dog	—	—	—	120	60	300

either of these substances with the activator which is already present in non-dialysed extract. Of course, the concentration of the activator in the non-dialysed extract is not optimal, so that if, for instance, citrate is added with some additional boiled juice, 410 μg./g. acetylcholine is synthesized, as compared with 230 μg. synthesized in the absence of boiled juice. These figures of 410 and 230 μg. acetylcholine refer to samples to which no ATP was added. The fact that ATP is not an irreplaceable component of the enzyme system which synthesizes acetylcholine emerges even more clearly from experiments with the dialysed brain extract (Table 2). The dialysis removes the activator from the extract so that the effect of ATP and citrate can be studied independently of the activator. Under these conditions, the quantities of acetylcholine formed in 1 hr. at 37° C. were as follows: with the dialysed extract alone 0 μg./g., in the presence of citrate 30 μg./g., in the presence of the activator 40 μg/g., in the presence of ATP 50 μg./g. It can be seen that none of the three factors, citrate, activator or ATP, has a strong effect on the formation of acetylcholine, if present by itself. Only if at least two of these substances are added simultaneously does a rapid formation of acetylcholine take place. The corresponding figures are 310 μg./g. with activator + citrate, 500 with activator + ATP, 680 with citrate + ATP. If all three factors, ATP, activator and citrate, are added together, the level of acetylcholine formation reaches the high figure of 1100 μg./g.

Apart from the evidence outlined above, there are other facts which point to different mechanisms of action of the three factors. For instance, glucose strongly inhibits the formation of acetylcholine in the presence of ATP, but has no such effect on the synthesis carried out in the presence of citrate and boiled juice. If citrate and ATP are added together, there is only a partial inhibition by glucose (Table 6). Malonic acid, on the other hand, causes no inhibition of acetylcholine formation in the presence of ATP; it has, however, some inhibiting effect on the synthesis carried out in the presence of either citrate alone or of citrate and activator together, but in the absence of ATP (Table 7).

TABLE 6. Effect of glucose on the formation of acetylcholine in saline extracts from acetone-dried rat's brain

Concentration of glucose in mg./c.c.	μg. acetylcholine formed in 1 hr./g. acetone powder in the presence of		
	ATP	ATP and citrate	Citrate and activator
0	400	900	370
0·2	40	550	380
1	40	520	350
3	40	550	380

TABLE 7. Effect of malonic acid on the formation of acetylcholine in saline extracts from acetone-dried brain in the presence of citrate and activator but in the absence of ATP

Animal	Molar concentration of citrate	μg. acetylcholine formed in 1 hr./g. acetone powder	
		Without malonic acid	With 0·02 M-malonic acid
Rat	0·00008	65	65
	0·00025	110	90
	0·0008	340	180
	0·0033	370	240
	0·01	360	230
Guinea-pig	0·01	180	90
	0·01	85	45*

* No activator added to samples.

As mentioned already, pyruvic acid strongly inhibits the formation of acetylcholine in a system which contains ATP (Table 4). An equally strong inhibition was observed if sodium pyruvate was added to a system containing no ATP. It was found that the formation of acetylcholine in a non-dialysed saline extract, to which citrate and activator had been added, was decreased by 0·002 N-pyruvate from 400 to 25 μg./g.

The enzymic formation of acetylcholine in extracts prepared from various parts of the nervous tissue

There are considerable differences in the synthesizing ability of the brains of various animal species, as assessed by experiments in which saline extracts were prepared from the acetone-dried brain tissue. In the absence of ATP, the rat brain extracts are the most active; the others follow in the order guinea-pig, rabbit, cat, dog (Table 5). In the presence of ATP the synthesizing capacity diminishes in the following order: guinea-pig, rat, rabbit, cat, dog, day-old kitten (Tables 1 and 5). The difference between the cat and kitten brain is still larger if the results are expressed in quantities of acetylcholine formed by 1 g. fresh brain tissue, instead of by 1 g. acetone powder, since the water content of the kitten brain is much higher than that of the adult cat (see Methods).

The synthesizing ability is not the same in all parts of the nervous system. In the central nervous system it is much higher in the cerebrum than in the

TABLE 8. Comparison of the acetylcholine content of nervous tissue with its ability to form acetylcholine

Animal and tissue	Acetylcholine content of fresh tissue calculated in: μg./g. wet tissue	μg./g. acetone-dried tissue	μg. acetylcholine formed in 1 hr./g. acetone-dried tissue in the presence of: ATP and citrate	ATP, citrate and activator
	Central nervous system			
Brain: Guinea-pig	3·6*	22·8	1000–1240	1450–1800
Rat	2·7	14·9	850–1160	1100–1200
Rabbit	1·8	9·9	600–720	1050–1280
Cat	1·2	5·8	480	580
Dog	0·1–0·3	0·48–1·44	300–380	360–480
Retina: Pig	(0·5)†	8·3	720	1170
Dog	—	—	—	400
Brain stem: Dog	0·8	3·8	—	420
Cerebral hemisphere: Dog	0·07	0·24	—	280
Cerebellum: Guinea-pig	0·2–0·4	1–2	210	290
Rabbit	0·06	0·3	150	200
Dog	0‡	—	—	40
Optic nerve: Dog	0‡	—	—	0–15
	Peripheral nervous system			
Posterior spinal roots: Dog	0‡	—	0–3	0–9
Anterior spinal roots: Dog	10–15	40–60	—	400–1240
Vagus fascicle: Dog	3	12	—	360

* From Feldberg (1944).
† In this case the wet tissue includes a large proportion of saline solution.
‡ Certainly less than 0·05 μg.

cerebellum; it is high in the retina but absent from the optic nerve (Table 8). In the peripheral nervous system, the anterior spinal roots show a great ability to form acetylcholine, whereas the posterior spinal roots hardly form any acetylcholine (Table 9). We envisaged the possibility that the posterior

TABLE 9. Formation of acetylcholine in saline extracts from acetone-dried tissue of the peripheral nervous system of the dog in the presence of ATP, citrate and the activator

Tissue	μg. acetylcholine formed in 1 hr./g. acetone-dried tissue
Posterior spinal roots	0, 0, 3, 7, 9
Anterior spinal roots	400, 470, 500, 640, 700, 850, 1050, 1240
Vagus fascicle	360, 600
Vago-sympathetic trunk	85, 230
Posterior root ganglia	12, 50

roots may gradually acquire a higher synthesizing capacity as they approach the spinal cord. To test this, the posterior roots (L_7, S_1, S_2) of a dog were each cut in the middle, so as to separate the parts which are near to the spinal cord from those which are close to the ganglion. In four experiments, the quantities of acetylcholine formed per g. acetone-dried nervous tissue were 0, 2, 8 and 9 μg. in the parts of the posterior roots which are near the spinal cord and 0, 3, 6 and 7 μg. in the parts adjoining the ganglia. Thus no evidence was obtained for an uneven distribution of the synthesizing system

in the two parts of posterior roots. Apart from the anterior roots, a fairly strong synthesizing ability was found in the vagus nerve of the neck; the acetone-dried preparation obtained from a fascicle of vagus nerve fibres, dissected out from the epineurium and from sympathetic fibres, was more active than a corresponding preparation obtained by acetone-drying of the tissue of the whole vagus trunk, including the sympathetic fibres.

We have also investigated the possibility of a correlation between the magnitude of the synthesizing capacity, as assessed by experiments on the acetone-dried tissue, and the content of acetylcholine as determined in the fresh tissue of the various parts of the nervous system. The results are recorded in Table 8. They show that there is a certain parallelism between the synthesis and the content of acetylcholine in the central as well as in the peripheral nervous tissue. There is, however, a difference between the central and the peripheral nervous tissue as shown by the following example. The content of acetylcholine per g. fresh tissue is 3 μg. in the vagus of the dog, 0·1–0·2 μg. in the dog's brain and 2·7 μg. in the guinea-pig's brain. The synthesizing ability, however, as assessed by experiments with acetone-dried preparations is 360 μg./g. acetylcholine in the vagus nerve of the dog, about the same amount (360–480 μg./g.) in dog's brain, but as much as 1100–1200 μg./g. in the guinea-pig's brain. It appears, therefore, that the central nervous tissue has, in comparison with the peripheral nervous tissue, a lower content of acetylcholine, but a higher capacity for acetylcholine synthesis.

DISCUSSION

The experiments presented above show that the activity of the enzyme which brings about the formation of acetylcholine is dependent on several thermostable and dialysable factors. Among the substances investigated there are three which have a particularly strong activating effect on the enzyme obtained by saline extraction of the acetone-dried nervous tissue. They are ATP, citric acid and the 'activator'. The activator is not identical with either choline, KCl, ATP, citric acid, glutathione or cozymase; nor is it identical with aneurin, since, as previously shown, aneurin has, if anything, an inhibiting effect on the formation of acetylcholine (Feldberg & Mann, 1945*a*). The activator can be conveniently prepared by extraction of the fresh or acetone-dried nervous tissue with boiling water or boiling saline solution. It should be pointed out, however, that non-dialysed enzyme preparations prepared by extraction of the acetone-dried tissue with cold saline solution also contain some activator as well as the enzyme. This fact partly accounts for the high synthesizing activity shown by non-dialysed enzyme extracts. There is substantial evidence that the activator is also involved in the synthesis of acetylcholine in brain slices and pulp, as well as in brain tissue dried, without acetone, in the desiccator and powdered (Feldberg, 1944).

The precise function of the activator cannot be defined until its chemical composition is established. It is also too early to speculate on the connexion which may exist between the activator and ATP. It is known that several substances become active as co-enzymes after they have been phosphorylated by ATP. Thus, aneurin forms co-carboxylase by reacting with ATP, and pyridoxal is phosphorylated by ATP to form the co-enzyme of the amino-acid decarboxylases. A similar mechanism may be involved in the synthesis of acetylcholine in the brain and nervous tissue. This would explain the fact that ATP, if added to the dialysed saline extract alone, is scarcely able to promote the formation of acetylcholine. On the other hand, there are several observations which suggest that the mechanism of the acetylcholine synthesis may be of a much more complex nature. There is, for instance, the fact that ATP added together with citric acid increases the synthesis of acetylcholine in dialysed brain extracts, even in the absence of the activator. Moreover, there is the observation that in both the non-dialysed and the dialysed brain extract the activator and citric acid, if added together, can induce a comparatively large formation of acetylcholine even in the absence of ATP.

In this paper the ability of the various parts of the nervous system to synthesize acetylcholine has been assessed by quantitative determinations on enzyme extracts prepared from the acetone-dried material. Such a method has its obvious limitations as compared with an assay carried out on fresh tissue. It has, however, certain advantages, and one of them is the great sensitivity of the method attributable to the very high and constant rate of acetylcholine formation in the acetone-dried tissue. With the new method it is possible to detect the formation of acetylcholine even in instances where other methods, such as those based on the use of fresh tissue, have given negative results. The method made possible a reinvestigation of the ability of various parts of the nervous system to synthesize acetylcholine and, in particular, the non-cholinergic (afferent) and the cholinergic (efferent) nerves. Afferent nerves, the optic nerve and the posterior spinal roots, for instance, have again been shown to be unable to form acetylcholine; at least the rate of formation never reached 20 μg./g./hr. Cholinergic nerves, such as the anterior spinal roots, on the other hand, may synthesize as much as 1240 μg. acetylcholine/g./hr. The non-cholinergic nerves had been shown previously (Feldberg, 1942–3) to be unable to synthesize acetylcholine, but the assays were carried out by means of relatively insensitive methods, and the argument has been advanced that the non-cholinergic nerves may have some synthesizing ability which is too small to be detected by these methods. This argument can hardly apply to the new method. Since, as shown above, the afferent fibres are practically devoid of the enzyme system which synthesizes acetylcholine, it is difficult, if not impossible, to see how they could form and utilize acetylcholine for the purpose of the initiation and propagation of nerve impulses.

There is thus no justification for the claims put forward from time to time (for references see Feldberg, 1945) that the release of acetylcholine is inseparably connected with the appearance and propagation of the nerve impulse. In order to uphold such views one would have to make an as yet unfounded assumption that the mechanism which underlies the formation of impulses varies according to the nature of the nerve fibres.

The inability of afferent nerve fibres to synthesize acetylcholine is also of interest in connexion with the theory that acetylcholine acts as the transmitter of nerve impulses at the central synapses. It is difficult to see how the nerve impulse may release acetylcholine at the sensory central synapse if the fibres lack the ability to produce acetylcholine. There is of course the possibility, as first suggested by Hellauer & Loewi (1938), that the fibre may be able to form acetylcholine only at its endings. Our observation is that all parts of the posterior spinal roots are almost completely lacking in the enzyme which synthesizes acetylcholine. It may be argued, however, that the majority of the fibres which compose the posterior spinal roots have their endings in the spinal cord far away from the point of entrance.

It is interesting to note that the retina has a great ability to synthesize acetylcholine. In this respect it closely resembles cholinergic nerve fibres, and the acetylcholine may act in a similar way in both cases. It may well be that acetylcholine is the chemical transmitter at one or more of the synaptic junctions in the retina. It should be emphasized that, in contrast to the retina, the optic nerve, originating in the ganglionic layer of the retina, does not synthesize acetylcholine.

SUMMARY

1. A study has been made of the mechanism involved in the enzymic synthesis of acetylcholine in the brain and nervous tissue. The enzyme was obtained by extraction of the acetone-dried tissue with saline solution (0·9% NaCl) and its activity studied in the presence of various thermostable factors. Among the factors investigated three greatly increased the activity, adenosinetriphosphate, citric acid and a third substance, referred to as the 'activator', which was found in the juice prepared by extraction of the brain tissue with boiling saline solution.

2. The activator is a dialysable substance. It is not a mineral constituent and it is not identical with either choline, ATP, glutathione, cozymase or aneurin. It is present in the liver in approximately the same concentration as in the brain. Smaller amounts occur in muscle and in yeast.

3. The activating effect of citric acid is not due to its Ca-binding capacity and is not abolished by malonic acid. None of the other organic acids examined had an accelerating effect comparable with that of citric acid.

4. If ATP, citrate and the activator are added together to the saline extract from acetone-dried brain, as much as 1800 μg. acetylcholine may be formed/g.

acetone powder/hr. at 37° C. This corresponds to about 6 mg. acetylcholine synthesized per g. dry material contained in the enzyme extract.

5. On dialysis, the saline extract from the acetone-dried tissue loses its ability to form acetylcholine. None of the three thermostable factors, if added alone, can promote the synthesis of acetylcholine to any appreciable extent in the isolated enzyme system. Full activity can be restored, however, by the addition of ATP, citrate and the activator. But even if ATP is omitted and only citrate and the activator added, then there is a large formation of acetylcholine.

6. The concentration of the enzyme system which synthesizes acetylcholine in the brain varies considerably from one species to another. In the same species there are great differences between the various parts of the nervous system. The synthesizing ability is greater in the cerebrum than in the cerebellum; it is high in the retina. Nerves consisting of afferent fibres only, e.g. optic nerve and posterior spinal roots, have hardly any synthesizing ability, whereas nerves consisting of efferent cholinergic fibres, e.g. anterior spinal roots, have a strong synthesizing power.

7. There is a definite relation between the rate of acetylcholine synthesis and the content of acetylcholine in the nervous tissue. The relation, however, is not the same in the central and peripheral nervous system. For a given rate of synthesis the corresponding content of acetylcholine is higher in the peripheral than in the central nervous system.

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CIRCULATORY CHANGES DURING FAINTING AND COMA CAUSED BY OXYGEN LACK

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It has recently been found that dilatation of the muscle blood vessels takes place in subjects who faint from loss of blood (Barcroft, Edholm, McMichael & Sharpey-Schafer, 1944; Barcroft & Edholm, 1945). This vasodilatation in muscle causes the acute drop in arterial blood pressure, the cerebral anaemia and the pallor of the skin. Further work was needed to find whether muscle vasodilatation was a constant manifestation of the vaso-vagal syndrome (Lewis, 1932), irrespective of how the syndrome was excited. It was therefore necessary to use some stimulus other than haemorrhage to elicit the fainting reaction. The stimulus chosen was hypoxia. Henderson (1938), Schneider (1918), Ershler, Kossman & White (1942) and Keys, Stapp & Violante (1942) have shown that moderate reduction in the oxygen in the inspired air induces fainting in some subjects. In other subjects hypoxia causes loss of consciousness without fainting, i.e. without the vaso-vagal syndrome. We therefore had an opportunity to study the blood flow in the forearm, which is mainly muscle, in both 'fainters' and 'non-fainters'. Abramson, Landt & Benjamin (1941) and McMichael & Snyder (1943) have investigated the blood flow in the forearm and in the leg during hypoxia, but not during fainting or coma.

Experiments of the effect of hypoxia in 'posthaemorrhagic' subjects are also described, as they throw further light on the nature of the vaso-vagal syndrome.

METHODS

The subjects were healthy men aged 20–30 years. Room temperature was 18–20° C.

The subject lay on a couch with his back supported at an angle of about 45°. Arrangements were completed for recording the blood flow in the left forearm by the Brodie venous occlusion principle, using the Lewis-Grant plethysmograph (Barcroft & Edholm, 1943). Water-bath temperature was 33–34° C. A sphygmomanometer cuff was put on the right arm. The subject was made comfortably warm with blankets.

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Pulse, arterial blood pressure, and forearm blood-flow determinations were made at 5 min. intervals. At 15 min. an oxygen mask (R.C.A.F. pattern) was put on and tested to see that it made an airtight fit. The subject breathed air from the room through the mask. At 20 min. air from a cylinder of compressed air was substituted. At 30 min. administration of a low percentage of oxygen in nitrogen was begun. Mixtures containing approximately 10, 8, 7 and 6% oxygen were available. Most experiments began with 8%. During the hypoxia, determinations were made of pulse, blood pressure and forearm blood flow at 3 min. intervals. After each reading the subject was shown a numbered photograph to test his mental condition, and his hands were examined for involuntary movements. After 15 min. of moderate hypoxia, a mixture poorer in oxygen was usually given.

(*a*) In a 'fainter', several determinations of pulse, blood pressure, and forearm blood flow (collecting pressure lowered to 30 mm. Hg) were made at about 1 min. intervals.

(*b*) In a 'non-fainter' hypoxia was continued till there were definite signs of depression of the higher centres; either deep stupor from which he could barely be roused, or total analgesia to pin-prick.

The mask was then taken off, and readings were continued for ½ hr. The subject was questioned about his sensations and his recollection of the photographs.

In the experiments on the posthaemorrhagic hypoxia a 6½ in. sphygmomanometer cuff was placed round each thigh, as high up as possible, and inflated to diastolic pressure at time 0. This procedure dams back blood in the veins of the legs and so produces the equivalent of a large haemorrhage, but not large enough to cause fainting, or only very rarely so (McMichael & Sharpey-Schafer, 1944; Ebert & Stead, 1940). At 15 min. the pressure was raised to 110 mm. Hg, and the mask was put on. At 30 min. 8% oxygen was given. After a faint, the administration of the hypoxic mixture was generally maintained for 15 min. or more. The procedure for a non-fainter was as above. At 60 min. the cuffs were deflated and the mask taken off in those subjects from whom this had not been done before.

RESULTS

There were three fainters and ten non-fainters.

Fainters. Fig. 1 shows typical results. Mask breathing barely altered the pulse, blood pressure or forearm blood flow. 9·8% oxygen was administered. In 2 min. the subject became slightly cyanosed. After 7 min. the pulse rate had risen to 120 beats/min., the blood pressure to 164/83 mm. Hg. The forearm blood flow was 3·1 c.c./100 c.c. forearm/min. Then there was a typical vaso-vagal faint. The subject was very pale. Profuse perspiration broke out. The radial pulse could no longer be felt. Consciousness was lost. The heart rate, measured by auscultation at the apex, had slowed to 56. The blood pressure was 85/75. The forearm blood flow had risen to 10·1 c.c./min., signifying a marked vaso-dilatation in the forearm. Then the mask was taken off. The blood pressure rose, the forearm flow subsided, and within 2 min. the subject was able to answer questions. Bradycardia and slight pallor persisted for many minutes. About 1 hr. after fainting the subject left the room feeling perfectly well.

Similar results were obtained in experiments on two other 'fainters'. During fainting there was an acute fall in blood pressure and an increase in forearm blood flow signifying vasodilatation. Fig. 2 shows the averaged results (peak flows in the other two experiments were 20 and 8·7 c.c.). There is no doubt that vasodilatation in the forearm occurs during hypoxic fainting.

Non-fainters. Fig. 3 shows a typical experiment. Breathing air through the mask caused no significant changes. 4 min. after 8% oxygen was given the subject was cyanosed, the pulse rate had risen to 125 and the blood pressure to 140/80; there was a slight rise in the forearm blood flow. After 10 min. of hypoxia the subject looked distressed, his arms and legs were twitching. He was deeply cyanosed but could still understand what was said to him. The 8%

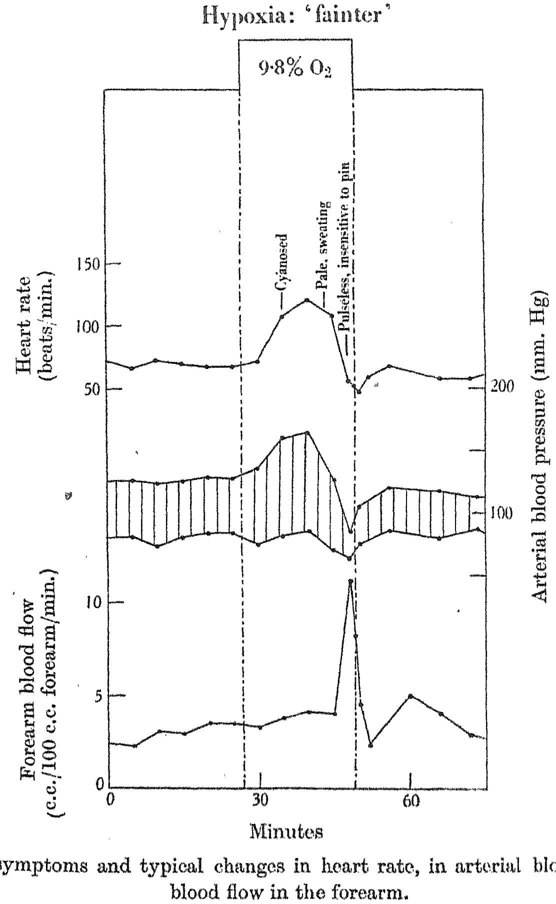

Fig. 1. Typical symptoms and typical changes in heart rate, in arterial blood pressure and in blood flow in the forearm.

oxygen was replaced by 7%. The subject soon lost consciousness and became quite insensitive to pin-prick. The pulse rate (110), high blood pressure (165/85) and rapid forearm flow (8·5 c.c./min.) showed that there was no sign of the vaso-vagal reaction. 2 min. after the mask was taken off the subject's colour was normal. He did not remember the removal of the mask. He had a headache. The pulse, blood pressure and forearm blood flow very quickly returned to normal. 75 min. later he left the room perfectly well except for a slight headache.

Fig. 4 shows the results of nine other experiments on non-fainters. The effect on the forearm blood flow varied from none in Exp. 2 to a sevenfold increase in Exp. 9.

It is noteworthy that involuntary muscular movements preceded coma in every experiment.

Fig. 2 shows the averaged results of the experiments on the non-fainters. It emphasizes the activity of the circulation during depression of the higher centres.

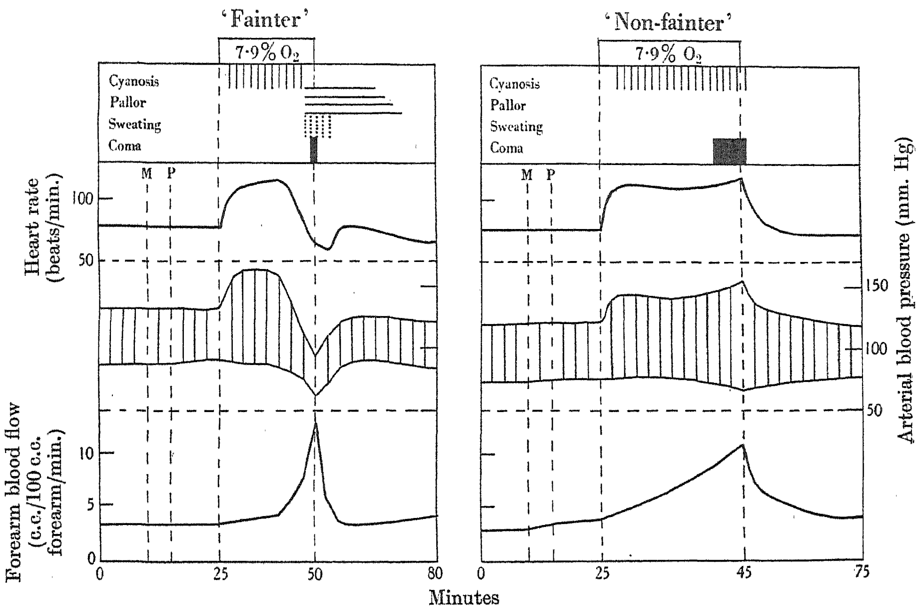

Fig. 2. Averaged results. *M*, mask put on, room air breathed. *P*, air from cylinder of compressed air breathed.

'Posthaemorrhagic' hypoxia. The same thirteen men who acted as subjects for the above experiments also acted for the experiments on the effect of hypoxia during the posthaemorrhagic state.

There was a striking increase in the proportion of 'fainters' to 'non-fainters' as is seen below:

	Fainters	Non-fainters
Hypoxia alone	3	10
'Posthaemorrhagic' hypoxia	10	3

The group of ten posthaemorrhagic fainters included the three subjects who had previously fainted during simple hypoxia.

A remarkable finding was that spontaneous recovery from fainting occurred in six subjects while they were still breathing the oxygen-poor mixture, and while they continued to have the blood dammed back in the legs. Fig. 5 shows

a typical experiment. Soon after beginning to breathe 8·4% oxygen, the subject became slightly cyanosed, the pulse rate rose to 114, and then there was a typical vaso-vagal faint. He became extremely pale and sweated. Consciousness was lost and pricking the skin elicited no response. The heart

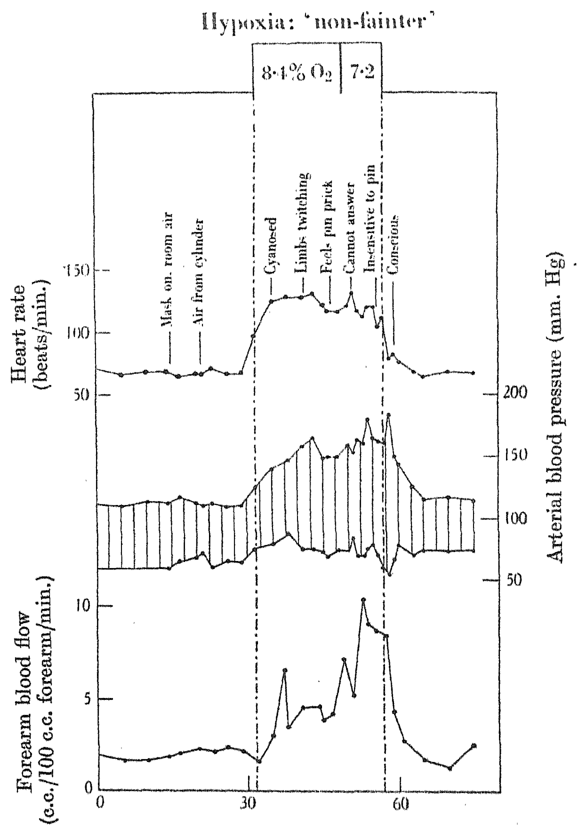

Fig. 3. Typical symptoms and typical changes in the heart rate, in the arterial blood pressure and in the blood flow in the forearm.

rate was 58, the blood pressure was 80/65, and the forearm flow had risen to 7·8 c.c./min. The blood was kept dammed back in the legs and the hypoxia continued. Within 2 or 3 min. the pulse rate and blood pressure started to rise. Spontaneous recovery had begun. 15 min. after fainting the subject had regained consciousness, was able to answer questions and was not distressed. The dilatation in the forearm had, to a large extent, subsided and the pulse rate and blood pressure had recovered their initial values and more.

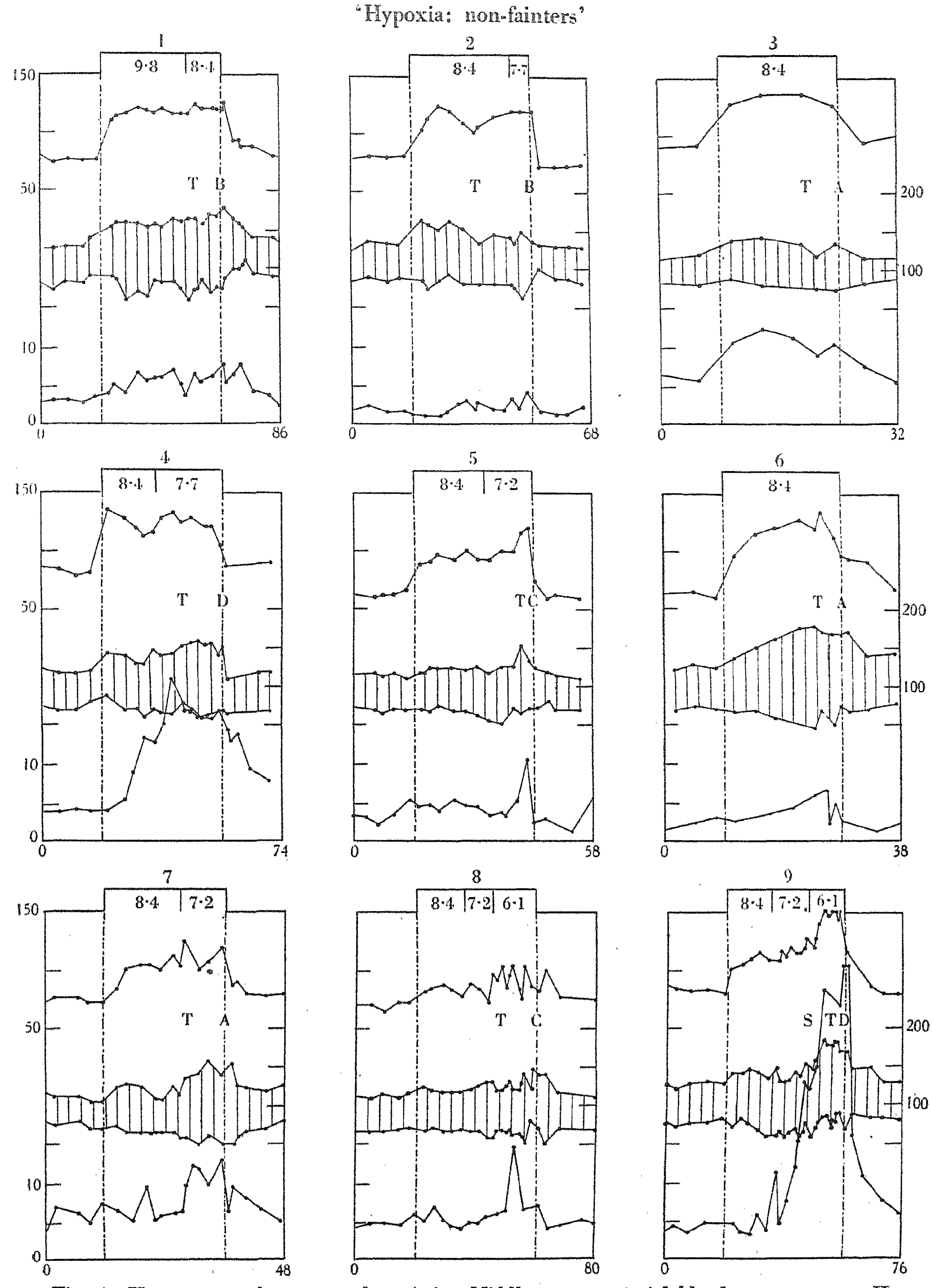

Fig. 4. Upper curve: heart rate, beats/min. Middle curve: arterial blood pressure, mm. Hg. Lower curve: forearm blood flow, c.c./100 c.c. forearm/min. Time in min. Raised portion of upper margin of graph: oxygen poor mixture breathed. The number under the upper margin is the percentage of oxygen. *T*, the onset of involuntary muscular twitches. *S*, the onset of involuntary muscular spasm. Mental condition at the end of the hypoxia: A, insensitive to pin-prick. B, could not be roused. C, could just be roused, amnesia. D, could just be roused, no amnesia.

DISCUSSION

The behaviour of the forearm blood flow in hypoxic coma differs somewhat from that found in lesser degrees of hypoxia. Abramson *et al.* (1941) found a 30% increase in forearm blood flow in subjects who breathed 10% oxygen.

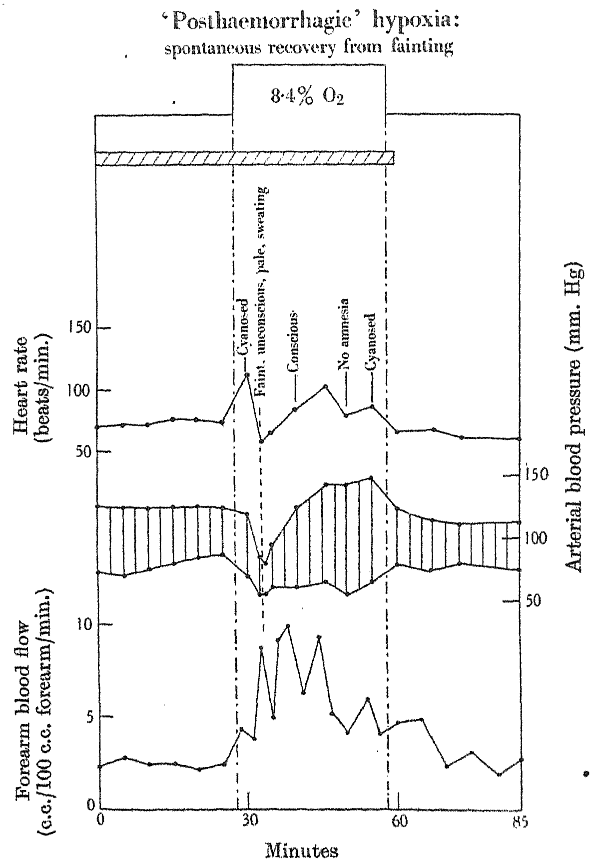

Fig. 5. Typical symptoms and typical changes in heart rate, in arterial blood pressure and in forearm blood flow. Shaded rectangle: pneumatic thigh cuffs inflated.

McMichael & Snyder (1943) found variable changes ranging from −45 to +77% in subjects in a low-pressure chamber at 16,000–18,000 ft. In our own experiments there was not much change in the forearm blood flow in some comatose subjects, in others there was hyperaemia, sometimes very marked; forearm blood flow increased on the average from 3·8 to 11·1 c.c.

The whereabouts of the hyperaemia in the forearms of the comatose subjects was not investigated. In the experiment in which the hyperaemia was most noticeable there was little doubt that it was in the muscle as there were

frequent coarse muscular twitches and muscular activity causes a marked increase in forearm blood flow. As involuntary muscular movements preceded coma in every experiment, it is possible that they caused the hyperaemia seen in most experiments. If the increase in the forearm blood flow, averaging 5·3 c.c./100 c.c. forearm/min., was in the muscle, and if a similar increase took place throughout the skeletal musculature, cardiac output would be increased by approximately 2·5 l./min. (for the basis of this estimate see Barcroft, Bonnar, Edholm & Effron, 1943). Possibly the increase in cardiac output found by Grollman (1930) and others in hypoxia may be mainly due to vasodilatation in the skeletal muscles.

As haemorrhage itself may excite fainting it is not surprising that the incidence of fainting due to hypoxia was increased in the subjects who had previously had a 'simulated haemorrhage'. The need for oxygen at comparatively low altitudes is suggested for wounded men who have lost much blood.

Ershler *et al.* (1942) found a rise in the pressure in the antecubital vein during fainting. They took this to be a sign of right ventricular failure. It seems, however, more likely that it is due to muscle vasodilatation and to increased forearm blood flow. The right auricular pressure is very little changed in fainting (Barcroft *et al.* 1944; Warren, Brannon, Stead & Merrill, 1945).

The view that hypoxic fainting was a heart failure was also held by Whitney (1918) who found that it was preceded by a large increase in the area of cardiac dullness to percussion. So far, X-ray pictures, made just before fainting, have shown no sign of enlargement of the heart (Keys *et al.* 1942). The remarkable recoveries from faints which took place in very adverse circumstances in the experiments described above do not suggest that there is heart failure during fainting.

SUMMARY

1. 7–8% oxygen was administered to thirteen normal subjects till three fainted and ten became comatose.

2. Heart rate, arterial blood pressure and forearm blood flow were determined.

3. *Fainters:* (*a*) During the faint there was pallor, sweating, bradycardia, acute drop in arterial blood pressure, marked increase in forearm blood flow and often loss of consciousness. (*b*) The following average values were obtained: heart rate, 62; arterial blood pressure 95/60 mm. Hg; forearm blood flow 13 c.c./100 c.c. forearm/min. (*c*) The finding of increased forearm flow in hypoxic as well as in posthaemorrhagic fainting indicates that active vasodilatation in skeletal muscle is a constant manifestation of the vaso-vagal syndrome.

4. *Non-fainters:* (*a*) During coma there was cyanosis, tachycardia, and usually rise in arterial blood pressure and increase in forearm blood flow. (*b*) The following average figures were obtained: heart rate, 120; arterial blood

pressure, 155/70 mm. Hg; forearm blood flow 11 c.c./100 c.c. forearm/min. (*c*) Possibly the increase in forearm blood flow and in cardiac output is caused, to some extent, by increase in the blood flow through the skeletal muscles.

5. Recovery from fainting occurred in six subjects while 8% oxygen was being administered and while about 1·5 l. of blood was kept dammed back in the legs. Recovery under such conditions does not support the view that fainting is a form of heart failure.

We express our warmest thanks to members of the Belfast Medical Students Association who acted as subjects.

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THE EFFECT OF ETHYL ALCOHOL AND SOME OTHER DIURETICS ON CHLORIDE EXCRETION IN MAN

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(*Received* 8 *November* 1945)

Evidence as to the nature of the diuresis following ingestion of ethyl alcohol (ethanol) suggests that it is similar to the diuresis following ingestion of water. In view of the lack of absolute proof of their identity, however, a further comparison of the two has been made in respect of certain properties by which a water diuresis can be differentiated from other types of diuresis. One such property is the response of the kidney in respect of excretion of chloride; an increased rate of urine flow is in general associated with an increased output of chloride, but, in a water diuresis, the great increase in rate of flow is accompanied by a diminished chloride output (Eggleton, 1943). The results of a series of class experiments on the effect of exercise on renal function, when different diuretics were used to promote urine flow, suggested that alcohol and water had the same effect in diminishing excretion of chloride (Eggleton, 1945), whereas tea increased it. The matter has now been investigated more fully and under more standardized conditions, without the additional effects of exercise.

METHODS

The general procedure was the same as that used in previous investigations. Each experiment was performed at least 5 hr. after the last meal, usually in the early afternoon; if in the morning, breakfast was omitted. Usually a glass of water was taken 2–3 hr. before the experiment began, to ensure a reasonable state of body hydration. In the experiments now reported, all substances under investigation were taken by mouth, and at least one urine sample was collected before their ingestion.

In a few of the earlier experiments, urinary chloride was determined by the electrometric titration method (Eggleton, Eggleton & Hamilton, 1937); in the remainder, a direct titration method, using tartrazine as indicator, was used with complete satisfaction. This method is simple and was found to be unexpectedly accurate over a wide range of chloride concentration in urine. The urine was delivered slowly from a 10 c.c. micro-burette into a boiling tube containing 2 c.c. 0·1 N-$AgNO_3$ solution, one drop of 2% tartrazine and 0·15 c.c. conc. HNO_3. At the end-point, the buff-yellow colour adsorbed on the precipitate changes to a greenish yellow colour diffused through the solution. Under these conditions, the range of chloride concentration encountered in the urine samples during the course of this research (10–250 m.equiv./l.) could be determined with an accuracy of ± 1%, although the titration values ranged from 0·8 to 20 c.c.

RESULTS

In a group of subjects from which data on the changes in urine pH following ingestion of water and of ethyl alcohol have been already published (Eggleton, 1945), chloride output was also determined in three individuals. The average results given in Fig. 1 indicate that, in these three, chloride output was diminished as greatly during the alcohol diuresis as during the water diuresis.

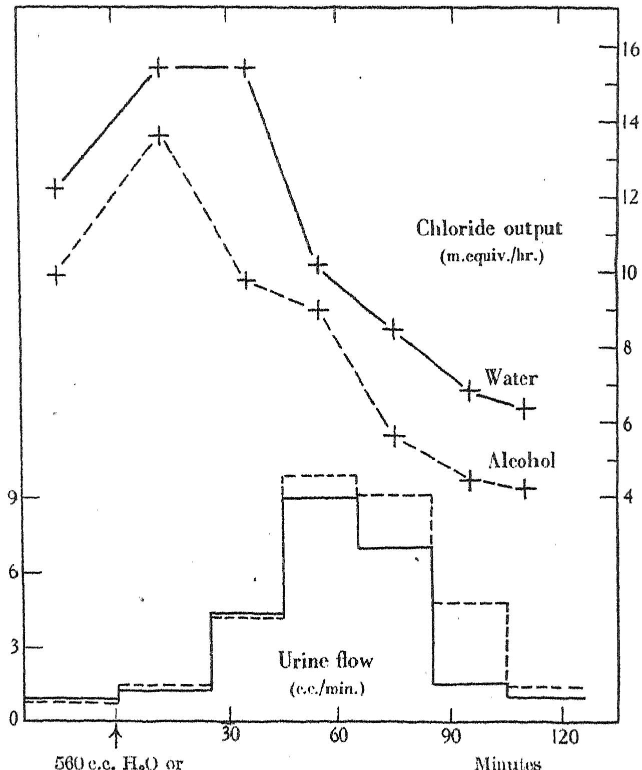

Fig. 1. The average changes in chloride output during water diuresis and during alcohol diuresis in three subjects. +——+ Water diuresis. +– – – –+ Alcohol diuresis.

Opportunity arose later, during an investigation into some further effects of alcohol, to extend the observations to a larger and different group of subjects. The average results obtained on the twelve subjects studied are shown in Fig. 2. There was a considerable individual variation in the degree to which the chloride output was decreased during alcohol diuresis, as had also been noted in connexion with water diuresis, but, in all but one subject, some diminution was observed. On the average, the chloride output, which rose with the first increase in rate of urine flow, fell again at the peak of

diuresis and later reached a value of 60% of the original resting value at a comparable rate of flow. This average final value ranged from 20 to 100% of the original; in no subject was any actual increase observed, apart from the characteristic early rise shown in Figs. 1 and 2.

This evidence is decisive as to the identical effects of water and of alcohol in promoting a diuresis with a diminished excretion of chloride; but its value in differentiating these two diuretics from all others depends on the accuracy of the commonly accepted fact that these others increase the output of chloride. Evidence in support of this concept is scattered and much of it is

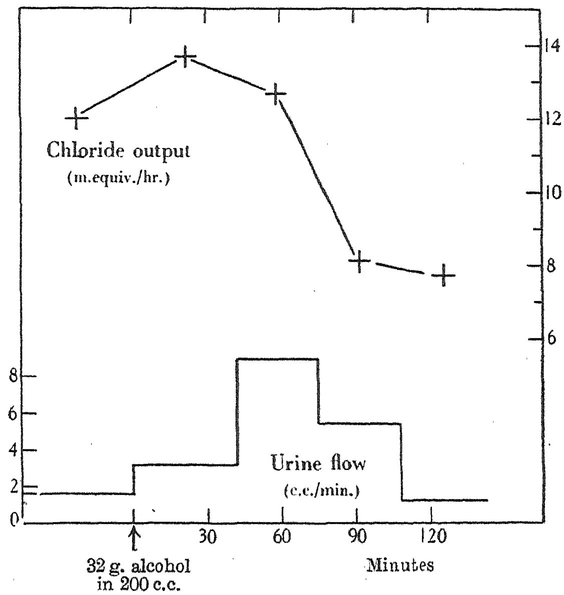

Fig. 2. The average change in chloride output during alcohol diuresis in twelve subjects.

derived from experiments on anaesthetized animals and on the isolated kidney. It seemed of interest, therefore, to attempt to demonstrate in an unanaesthetized subject the opposing actions on chloride excretion of water and of alcohol on the one hand, and of a series of typical diuretics on the other.

The experiments were carried out under the usual standard conditions over a period of 3–4 months. No attempt was made to standardize the diet during this period, but the general regime did not suffer any major changes. Variations in atmospheric temperature and humidity were great during the period in question, and were probably largely responsible for the variable rate of urine flow encountered in the resting sample on different experimental days. The results given in Table 1 suggest that this variable rate of flow was the major factor responsible for variations in the value of the resting chloride output.

TABLE 1. The relationship between rate of urine flow and chloride output under resting conditions in one subject

Date	Rate of urine flow c.c./min.	Chloride output m.equiv./hr.
3. v. 45	1·87	14·9
12. iii. 45	1·82	13·2
24. v. 45	1·45	13·4
15. iii. 45	1·4	10·8
14. vi. 45	0·93	8·3
7. v. 45	0·9	7·4
21. vi. 45	0·66	5·9
22. iii. 45	0·6	4·7

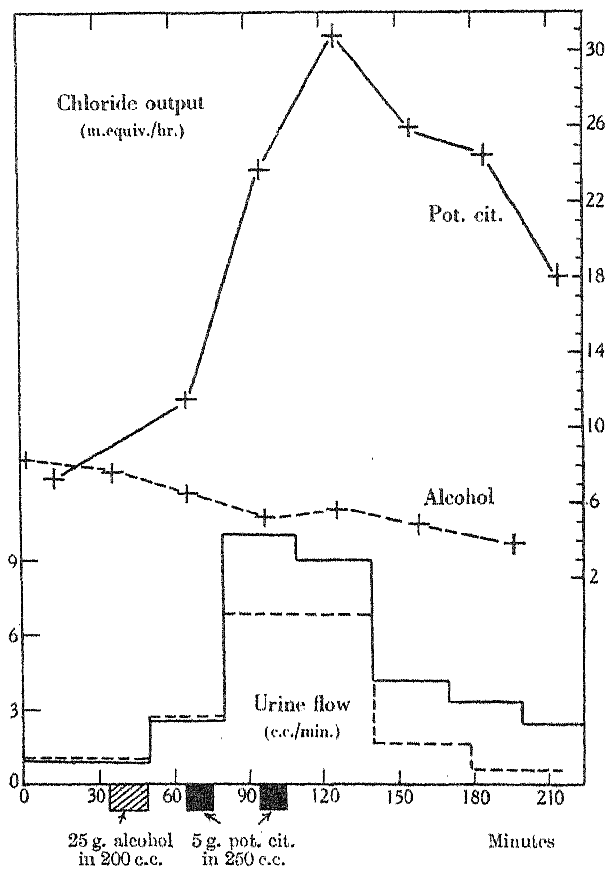

Fig. 3. A comparison between the chloride outputs during diureses of the same magnitude induced by alcohol and by potassium citrate. +——+ Potassium citrate. +– – – –+ Alcohol.

In addition to experiments with water and with ethyl alcohol, the effects of the following substances were examined: caffeine, urea, potassium citrate and glucose. It may be said at once that any diuresis resulting from ingestion of these substances was invariably accompanied by an increase, usually a large

increase, in excretion of chloride. In Fig. 3 the results obtained after ingestion of potassium citrate have been compared with those after ingestion of alcohol, since the starting-point, as regards rate of flow and chloride output, was practically identical on the two occasions, and the diuretic actions comparable in magnitude. This subject showed less depression of chloride output after alcohol than the average: at the height of diuresis chloride excretion fell from 8·4 to 5·4 m.equiv./hr. In comparison, the rather larger diuresis after ingestion of 10 g. potassium citrate in 500 c.c. water was accompanied by an increase in chloride output from 7·4 to 30·8 m.equiv./hr.

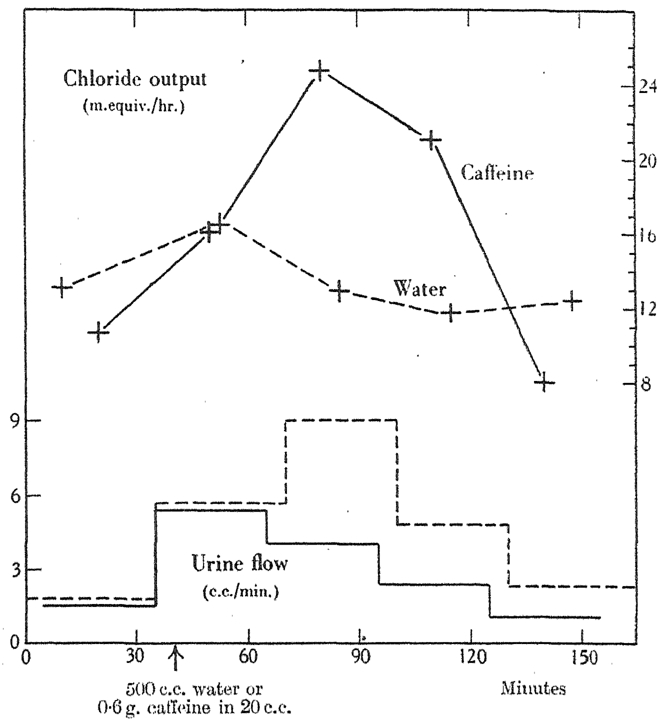

Fig. 4. A comparison between the chloride outputs during caffeine diuresis and during water diuresis. +——+ Caffeine. +- - - -+ Water.

Similarly, in Fig. 4, there can be seen the slight depression of chloride output accompanying a large water diuresis, and a rise from 11 to 25 m.equiv./hr. chloride with the smaller diuresis occasioned by ingestion of 0·6 g. caffeine in 20 c.c. water. Urea (32 g. in 200 c.c.) gave a response similar to that of caffeine, the chloride output rising from 8·7 to 22 m.equiv./hr., and in this experiment, as in those depicted in Figs. 3 and 4, the peak of chloride excretion lagged behind the peak of the diuresis. Glucose ingestion (200 g. in 220 c.c.) gave rise to no appreciable diuresis nor to any great rise in chloride output:

the flow rose only from 0·66 to 1·0 c.c./min. during the height of glycosuria and the chloride output increased from 5·9 to 7·3 m.equiv./hr. just after the peak of diuresis.

Further experiments were performed on the group of subjects on whom the results shown in Fig. 2 were obtained; these provided some evidence concerning the closeness of association between the chloride-depressing and the diuretic actions of alcohol, since, in the case of water diuresis, it has been suggested that two distinct mechanisms may be involved. In one subject (not included in the average shown in Fig. 2), ingestion of alcohol produced practically no diuresis, nevertheless there was a typical fall in chloride excretion (Fig. 5). The alcohol was undoubtedly absorbed: it was noted at the time as having a prolonged moderate action on the higher nervous centres; but the slow rate

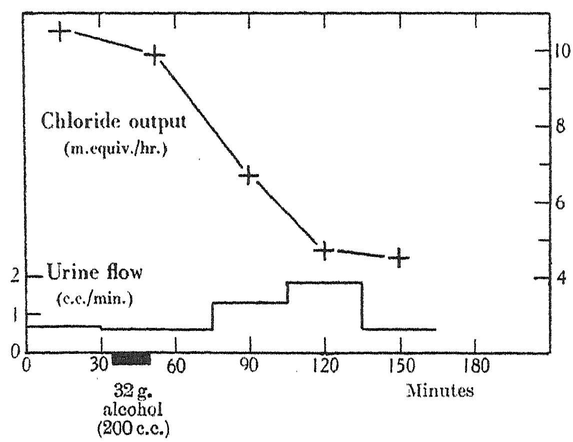

Fig. 5. Showing the suppression of chloride output following ingestion of alcohol in one subject when the diuresis was inhibited for an unknown reason.

of increase in blood alcohol concentration, probably resulting from slow and erratic emptying of the stomach, may have been insufficient to affect a rather insensitive pituitary mechanism. Such a state of affairs was encountered on a previous occasion (Eggleton, 1942) and in that subject also the pituitary mechanism was insensitive, i.e. there was an unusually small diuretic response to ingestion of water. In the subject used in the experiment illustrated in Fig. 5, 500 c.c. water was followed by a diuresis of only 355 c.c.; again, however, the usual fall in chloride output was observed, from 14·4 to 5·1 m.equiv./hr. (at identical rates of urine flow). In several subjects, subcutaneous administration of nicotine (1 mg. in the form of tartrate) before ingestion of alcohol almost suppressed the diuresis. It can be seen from their average results, shown in Fig. 6, that the typical decrease in chloride output nevertheless occurred. In other subjects, nicotine enhanced the diuresis, but the typical diminution of chloride excretion resulting from ingestion of alcohol

was again observed. The conclusion that nicotine itself cannot be held responsible for the decrease in chloride output is strengthened by the further observation that the magnitude of this decrease in the twelve subjects studied was of the same order, whether the alcohol was preceded by nicotine or not. A detailed report of these nicotine experiments will be given elsewhere.

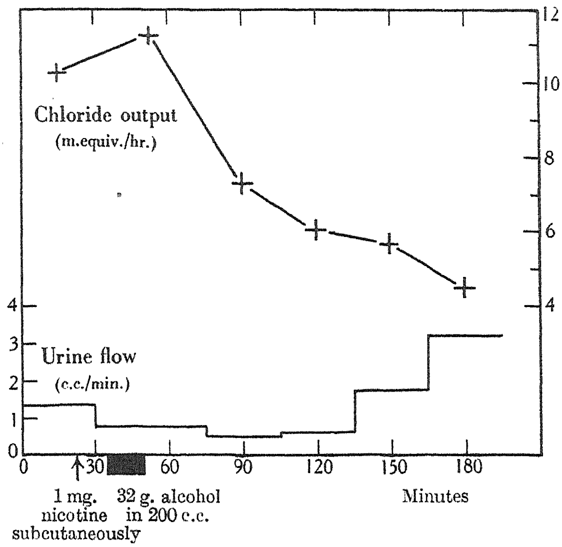

Fig. 6. Showing the average suppression of chloride output in four subjects following ingestion of alcohol when the diuresis was inhibited by nicotine.

DISCUSSION

It has been demonstrated clearly that ethyl alcohol shares with water the property of promoting a diuresis with diminution of chloride excretion, in contrast to such other and varied diuretics as caffeine, potassium citrate and urea, which promote a large increase in chloride output. Of the mechanism responsible for this differentiation between the two groups, little fresh evidence has come to light. It is now generally agreed (Goodman & Gilman, 1940) that the diuretics in both groups act by diminishing water reabsorption in the tubules, though caffeine and alcohol may also increase the glomerular filtration rate in the early stages. If this view of the action of diuretics in general be accepted, they might all be expected to cause an increased output of chloride, as part of the general 'flushing-out' process accompanying a faster rate of flow. There is now considerable doubt, however, as to the validity of this conception in the whole animal as distinct from the isolated kidney preparation. Various other explanations of the increase in chloride output after ingestion of caffeine and of potassium citrate are equally tenable, and it has been shown (McCance & Young, 1944) that increase in chloride excretion after

ingestion of urea may, in some circumstances (dehydration), be lacking in spite of an increase in rate of urine flow.

Thus, many factors can apparently override the simple 'flushing-out' process. Until conclusive evidence concerning the mechanism responsible for increased chloride reabsorption during water diuresis is forthcoming, the view that some pituitary factor, associated with the anti-diuretic hormone, is concerned remains the most probable. The fact that ethyl alcohol initiates the same chain of events, i.e. a large diuresis after a 20-30 min. lag accompanied by a decreased output of chloride, suggests that the two factors, antidiuretic and chloride-reabsorbing, are closely linked. That they are not identical has been already suggested in the case of water diuresis (Eggleton, 1943), corroborating an earlier conclusion of Fee (1928) on dogs. He found that inhibition of a water diuresis by various anaesthetics did not prevent the typical fall in chloride output normally accompanying the diuresis. Later, Bayliss & Brown (1940), working on hypophysectomized decerebrate dogs, were led to the tentative conclusion that some structure in the tuber cinereum was concerned with the excretion of chloride.

The fact that a similar dissociation of the two responses of the kidney, in regard to the excretion of water and of chloride, can be observed when the diuresis is stimulated by alcohol in place of water is again strongly suggestive that the two agents are working through the same intermediary mechanism.

SUMMARY

1. The depression of chloride output in the urine accompanying water diuresis is observed also during alcohol diuresis (Figs. 1, 2).

2. Comparison, in the same individual, of these two diuretics with others shows that they are unique in this respect. Potassium citrate (Fig. 3), caffeine (Fig. 4) and urea all produce a large increase in chloride output during the diuresis.

3. The decrease in chloride output after ingestion of alcohol, as in that after ingestion of water, is not closely correlated with the degree of diuresis and may be observed when this is almost completely suppressed (Figs. 5, 6).

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THE OSMOTIC PRESSURE OF HUMAN FOETAL AND MATERNAL SERA

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(*Received* 12 *December* 1945)

Previous work has shown that foetal sheep serum exerts a higher osmotic pressure per gram of protein than maternal serum (McCarthy, 1938). Since this finding could only partially be explained by a difference in the albumin/globulin (A/G) ratios of the sera, osmotic-pressure measurements of foetal horse serum crystalbumin were made. The albumin of foetal horse serum, which was chosen in preference to the albumin of foetal sheep serum as it is readily crystallizable, showed no significant difference in molecular weight from that of adult horse serum albumin. Assuming that foetal and maternal albumins in the sheep have the same molecular weights, the higher colloidal osmotic pressure of foetal sheep serum was attributed to the globulin fraction (McCarthy, 1942).

Macheboeuf (1929) and Macheboeuf & Sandor (1931) reported that marked alteration in the lipid content significantly affects the osmotic pressure of serum protein. Since the lipid content of human and sheep foetal serum is lower than that of the maternal (Slemons & Stander, 1923; Barcroft & Popják, 1944), the possible influence of lipids on the serum osmotic pressure was investigated. The results indicated that the lipids removed from normal human serum by McFarlane's method (McFarlane, 1942) exerted no effect on the osmotic pressure of the serum proteins (Popják & McCarthy, 1943).

Pedersen (1944) prepared a protein, Fetuin, with a molecular weight of about 50,000, from foetal blood of sheep and other species, including man. This fraction of low molecular weight constitutes 48% of the total serum globulin in the foetal sheep but only 2% of the globulins in human umbilical cord blood. This finding may explain the results of osmotic-pressure measurements on foetal sheep serum protein.

The present communication records osmotic-pressure measurements on human sera obtained from mothers and newborn babies. Some electrophoretic observations on the sera are also included.

OSMOTIC PRESSURE MEASUREMENTS

Umbilical cord blood samples were obtained at six births. The maternal blood samples were obtained by venepuncture within 2 hr. after delivery. The bloods were allowed to clot, and in four cases the sera were removed and placed in collodion membranes and dialysed against M/15 phosphate buffer mixture (M/30 $Na_2HPO_4 12H_2O$ + M/30 KH_2PO_4) of pH 6·8. Measurements of osmotic pressure were made at 0° C. with the same phosphate buffer mixture (Adair, 1925). Total protein concentrations were estimated by Kjeldahl nitrogen determinations. The ratio of albumin to globulin was obtained by saturation of the more concentrated solutions with magnesium sulphate and estimation of albumin nitrogen in the filtrate. The factors used for the calculation of protein concentrations were based on the figures for horse serum albumin, 15·60% N, and total globulin, 15·13% (Adair & Robinson, 1930*a*).

Table 1 records the proportions of albumin and total globulin in the sera of four mothers and their babies. The estimations of total protein in the fresh undialysed sera shown in Table 1 were made with the dipping refractometer

TABLE 1

Case no.	Source	Total protein* g./100 ml. serum	Percentage of albumin	Percentage of globulin
1	Mother	—	52·2	47·8
2	,,	7·1	48·0	52·0
3	,,	7·7	54·7	45·3
4	,,	6·8	50·3	49·6
1	Baby	—	56·4	43·6
2	,,	6·5	56·5	43·5
3	,,	6·0	64·7	35·3
4	,,	6·4	63·3	36·7

* Refractometric estimations (see text).

and represent very approximate values, since the serum ultrafiltrate was assumed in each case to have the refractive index of a 0·15 M-NaCl solution, and the effect of lipoproteins on the refractometer readings was unknown. The high foetal and the low maternal A/G ratios obtained are in general agreement with those described by Longsworth, Curtis & Pembroke (1945). Fig. 1 shows the protein osmotic pressure of four foetal sera in mm. Hg at 0° C. Similar data on the corresponding maternal sera are presented in Fig. 2. Comparison of the foetal and maternal sera may be effected by determining for both sera the values of π_0; i.e. the limiting value of the ratio $\frac{P\ \text{(osmotic pressure)}}{C\ \text{(concentration)}}$, in the infinitely dilute solution. Observed values of $1/\pi$ were plotted against C or protein concentration as abscissae. The points obtained approximate to a straight line, the equation of which was derived by the method of least squares. Since the observed differences in the osmotic pressure in each group were assumed to be due to experimental errors only, an equation expressing the

relationship between $1/\pi$ and C for each group was obtained. The relationship between $1/\pi$ and C for the four maternal sera was $1/\pi = -0{\cdot}0375C + 0{\cdot}56$, and for the foetal sera $1/\pi = -0{\cdot}0431C + 0{\cdot}56$. The value of $1/\pi_0$ or $1/\pi$ at infinite dilution is therefore 0·56 for both maternal and foetal sera and π_0 is 1·79.

The continuous curves in Figs. 1 and 2 were constructed from data calculated from the equation $p = \pi_0 C/(1 - K_b C)$ (Adair, 1928). The interrupted curve in

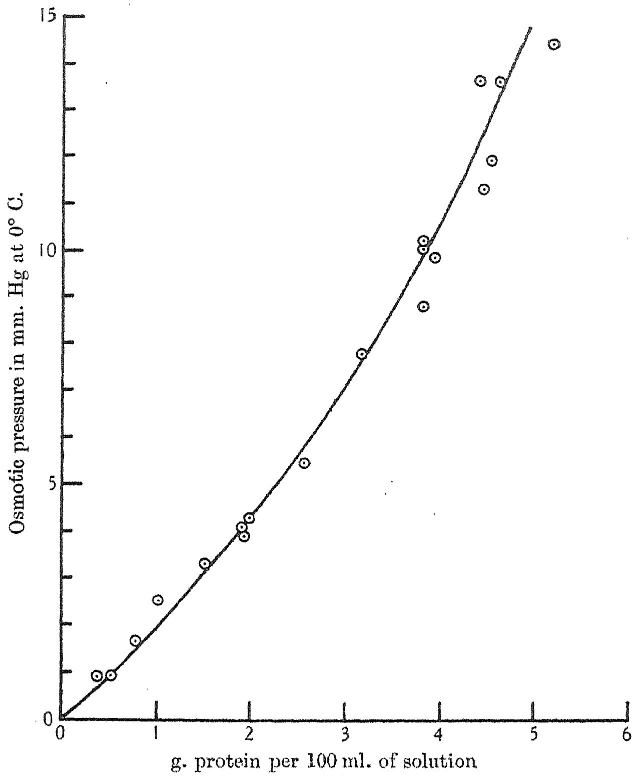

Fig. 1. Relationship between protein concentration and osmotic pressure of foetal sera. The data for the continuous curve have been calculated from the equation of Adair (1928): $p = \pi_0 C/(1 - K_b C)$ (cf. Adair & Robinson, 1930*b*). The circles represent observed values for four foetal sera.

Fig. 2 corresponds to the continuous curve in Fig. 1. Foetal and maternal serum proteins appear to exert the same osmotic pressure at low protein concentration. The slight difference which is observed at higher concentrations is due to different K_b values in foetal and maternal sera of 0·078 and 0·068 respectively. The significance of this finding is doubtful in view of the lack of precision in the observations. This must be in part, at least, attributable to limited quantities of material and uncontrollable fluctuations in temperature during the course of the experiments. The results in general seem to justify

the conclusion that the differences in serum protein osmotic pressure of foetal and maternal blood at birth which are so marked in the sheep are insignificant or absent altogether in man. The fact that Fetuin constitutes only 2% of the total globulin in human umbilical cord blood seems significant in this connexion.

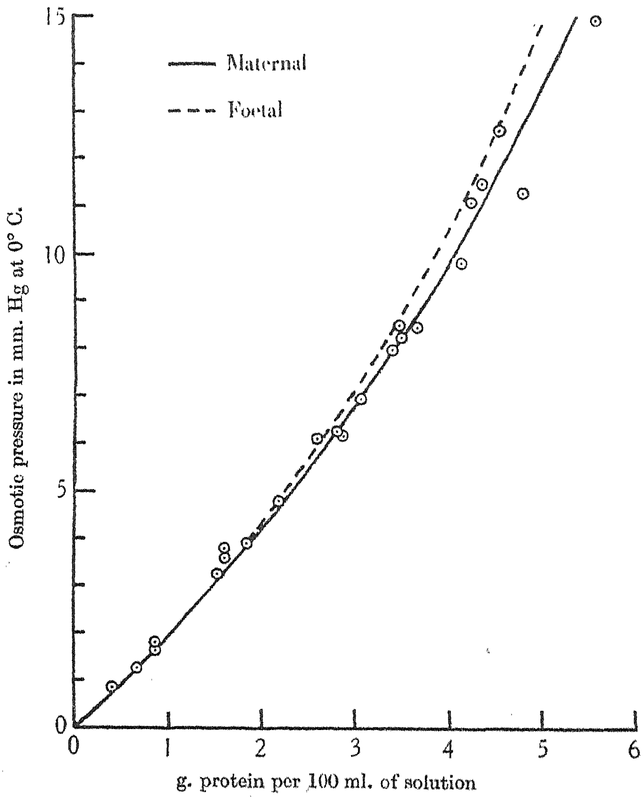

Fig. 2. Relationship between protein concentration and osmotic pressure of maternal sera (smooth curve). The foetal curve (interrupted curve) of Fig. 1 has been superimposed for purposes of comparison. Data for the curves have been calculated as described in the legend of Fig. 1. The circles represent the observed values for four maternal sera.

ELECTROPHORESIS

Sera obtained from two cases were used for electrophoretic analyses. The sera were dialysed for 3 days against phosphate buffer, of ionic strength 0·2 and pH 8. The dialysed sera were centrifuged at low speed for 5–10 min. to remove suspended material. The refractive indices due to protein R' and dialysate R'' were determined. The sera were diluted to an appropriate value of $R'-R''$ and analysed in the Tiselius (1937) apparatus. Optical observations were made with the diagonal Schlieren method (Philpot, 1938) using monochromatic

light $\lambda = 546\,m\mu$. (isolated from a high-pressure mercury arc by a suitable filter). Electrophoresis was continuous for 5 hr. at 0° C., with a potential gradient of 5 V./cm. The relative proportions of the serum-protein fractions observed were calculated. No measurements of migration velocities were made.

TABLE 2

			Percentages of components in sera		
				Globulins	
Case no.	Source	$R' - R''$	Albumin	$\alpha+\beta$	γ
5	Mother	0·00232	32·9	56·3	10·8
6	,,	0·00382	48·7	33·8	17·4
5	Baby	0·00235	60·8	17·4	21·8
6	,,	0·00374	64·8	26·8	8·4
	Normal*	—	60·3	23·9	11·0

* Mean values for fifteen normal adult plasmas determined by Dole (1944). The corresponding serum values for β and γ globulins, which should show a 5 % increase after the loss of fibrinogen, were somewhat higher than expected.

The work of previous investigators on the concentration of protein components in human foetal and maternal sera has been summarized and extended by Longsworth *et al.* (1945). Electrophoretic analyses of sera from two cases, Nos. 5 and 6, are recorded in Table 2. The δ boundary was excluded in computing the percentages on the ascending side. Patterns obtained from the

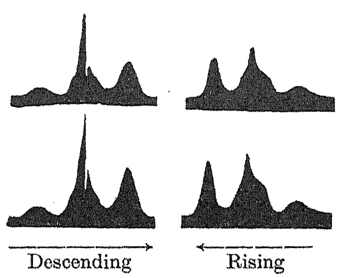

Fig. 3. Electrophoretic patterns of maternal serum No. 5.

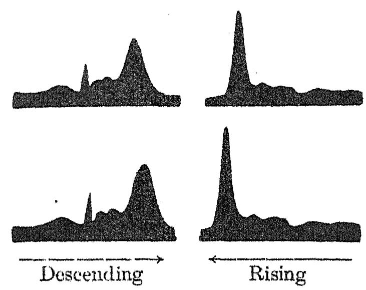

Fig. 4. Electrophoretic patterns of foetal serum No. 6 (see text).

descending limb did not show ϵ boundaries. Areas from both sides were used for the calculation of average values of all components except those of maternal serum No. 5. The latter represent values obtained from the cathode side only, as the δ boundary and γ globulin peak were not separable on the anode side. Mean values for normal plasmas determined by Dole (1944) are included for comparative purposes. Electrophoretic patterns of maternal serum No. 5 and foetal serum No. 6 are shown in Figs. 3 and 4 respectively. The observed increase in the maternal $\alpha+\beta$ component is of interest since a large portion of serum lipid is carried by this fraction (Blix, Tiselius & Svensson, 1941), and maternal serum is known to have a high lipid content (Boyd, 1934, 1936).

Electrophoretic analyses of human foetal and maternal sera from ten cases by Longsworth *et al.* (1945) have shown a relative and absolute increase above normal in foetal globulin and more markedly in maternal α and β globulins. Absolute concentrations are not recorded in Table 2 since the refractometric measurements of total protein in the undialysed sera yield very approximate values. The relative concentrations of serum components in No. 5 support the general conclusions of Longsworth *et al.* The γ globulin percentage of foetal serum No. 6 is lower, however, than that of the corresponding maternal serum.

SUMMARY

1. There is no significant difference in the colloidal osmotic pressure of human foetal and maternal serum. This finding is in sharp contrast to the previous observation that foetal sheep serum proteins exert a higher osmotic pressure than the maternal. It is suggested that this species difference is due to the high Fetuin content of foetal sheep serum.

2. Electrophoretic analyses of two pairs of human foetal and maternal sera are recorded.

I wish to thank Dr R. A. Kekwick and Dr B. R. Record for assisting in the electrophoretic experiments, and also Dr G. Popják for his help in presenting the osmotic pressure results.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY

10 *February* 1945

Relation between fibre diameter and action potential of single nerve fibres.

By Helge Hertz. *Theory of Gymnastics Laboratory, Copenhagen and Physiological Institute, Lund, Sweden*

Experiments have been made to find to what extent the propagation of the nervous impulse is dependent on (*a*) ionic exchange through the nerve membrane and (*b*) processes on the surface of the membrane.

Single fibres of the frog's sciatic were stimulated with brief condenser discharges, and monophasic potentials were recorded with a push-pull amplifier and cathode-ray oscillograph. The diameter of the fibre was measured with an eyepiece micrometer and water-immersion objective.

Osmotic experiments. Solutions, made up to 7 times hypertonic with NaCl or glucose, or $\frac{1}{3}$ hypotonic, were without effect on either diameter or action potential in the first 10 min. of exposure, showing the extreme water impermeability of the nerve membrane. Later, the nerve shrinks or swells by 10 %, and the declining phase of the action potential becomes progressively slower, until in $\frac{1}{2}$ hr., although the nerve has returned to its previous diameter, the negativity persists indefinitely after a stimulus. The recovery process had ceased on account of permanent damage to the fibre. The osmotic damage is only reversible if exposure is less than 5 min.

Effect of KCl. In contrast to the above experiments, immersion of the nerve in isosmotic solutions with a KCl content 6–10 times normal, causes an immediate and reversible reduction in the amplitude of the action potential (cf. Cowan, 1934); its shape remains normal. The effect of KCl can be only partially counteracted by an equivalent increase in the $CaCl_2$ concentration. An attempt to remove K^+ from the nerve by Na-Permutit ion-exchanger was without effect.

Other ions. Removal of Ca^{++} by citrate or oxalate did not cause spontaneous activity until $\frac{3}{4}$ hr. had elapsed. Magnesium chloride (200 mg./100 c.c.) was without effect over 2 hr., and so was acetylcholine chloride (1 mg./100 c.c.).

The experiments demonstrate the impermeability of the membrane around the axis cylinder, and it appears unlikely that any great exchange of ions could take place in the short time required for the action potential to reach its

maximum. The immediate effect of KCl shows that it acts on the surface, but a normal $K^+ : Ca^{++}$ ratio within the membrane is necessary for the surface phenomenon.

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The colour of small objects. By H. Hartridge

In a letter to *Nature*, E. N. Willmer suggested that the rod-free centre of the human fovea centralis is to some degree blue-blind. I have recently performed experiments which indicate the complexity of the problem.

Blue-blindness may be caused (I) by yellow-orange pigmentation either of the macula or of one of the eye media, or (II) by an absence of the blue sensation. The former type may be imitated experimentally and the visual defects observed directly. The latter type cannot be imitated, and in consequence the visual defects have to be inferred from the shapes of the sensation curves for red and green. In the following table the visual responses for these two types of blue-blindness are compared with those observed with the fovea at visual angles of 10, 4 and 1 min. of arc.

Actual colour	Blue-blindness		Foveal vision		
	Type I	Type II	10	4	1
Red	Red	Red	Red	Red	Red
Orange	Orange	Pink	Orange	Orange	Orange
Yellow	Yellow	White	Yellow	Pale yellow	White
Green	Green	Pale blue-green	Green	Pale green	Pale blue-green
Blue-green	Dark blue-green	Blue-green	Blue-green	Blue-green	Blue-green
Blue	Black	Dark blue-green	Blue	Dark blue	Dark grey
Violet	Black	Very dark blue-green	Violet	Dark violet	Black
Purple	Red	Pink-grey	Purple	Purple	Red-brown
White	Yellow	White	White	White	White

If the columns be compared it will be seen that foveal vision, which at 10 min. of arc should fall entirely within the rod-free area of the fovea, is practically normal in colour appreciation. At a visual angle of 4 min. of arc defects show themselves, but these do not correspond to blue-blindness either of types I or II. At the very small visual angle of 1 min. of arc, very serious defects are found, but it is possible that these are, in part, due to scattered light from other parts of the visual field. It should be emphasized that during all these experiments care was taken to preserve light adaptation everywhere over the retina.

Fat embolism due to the action of *Cl. welchii* toxin. By J. J. Elkes* and A. C. Frazer.* *From the Pharmacology Department, University of Birmingham*

Lecithinase of *Cl. welchii* type A toxin (Macfarlane & Knight, 1941) causes flocculation of chylomicrons due to interference with the stabilizing phospholipid film (Elkes & Frazer, 1944). It also causes breakdown of soluble plasma lipoprotein complexes (Nagler, 1939). It seemed possible, therefore, that this interference with blood-fat stability might result in fat embolism. To investigate this possibility, the effects of the toxin on various tissues have been studied *in vitro*, in experimental animals and in clinical cases of clostridial infection.

In vitro. Freshly obtained tissue or fluid substrates were incubated aseptically for 16 hr. with toxin (30 Lv. units α toxin/ml.)†; toxin-antitoxin controls were used as a basis of comparison. Muscle showed a marked reduction in lipoid phosphorus and liberation of free fat; adipose tissue was fragmented, freeing large masses of fat globules. Human serum showed typical Nagler reaction and flocculation of chylomicrons, but guinea-pig's serum showed neither of these changes.

Animal experiments. Guinea-pigs were injected intramuscularly with 60 Lv. units of toxin, diluted in 1 % procaine to obviate pain, and killed 1, 2, 4, 6, 16 or 24 hr. later. The local changes confirmed the findings of other workers (*M.R.C. Report*, 1919) and our *in vitro* observations. Abundant free fat could be seen in the oedema fluid. Frozen sections of the lung showed arteriolar fat emboli within 1 hr. of injection. Lesser degrees of fat embolism were demonstrable in other organs (myocardium, kidneys and spleen).

Clinical studies. Pulmonary fat embolism was demonstrable histologically in three fatal cases of clostridial infection. Sputum stained for fat by Robb-Smith's method was positive in one case which survived.

The embolic fat may be derived from three possible sources: the breakdown of fatty tissue at the site of injection, flocculated chylomicrons, or the splitting of the plasma lipoprotein complexes. Only the former occurs in the guinea-pig, and must therefore account for fat embolism in these experiments. In man both flocculation of chylomicrons and splitting of plasma lipoprotein complexes were seen *in vitro*, and these cannot be excluded as possible contributory factors in our cases.

* Sir Halley Stewart Research Fellow.

† An Lv. unit is a combining power unit, and equivalent to one Lf. unit (W. E. van Heyningen, private communication.)

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Placental growth after foetal death in the rat.

By A. St G. Huggett and J. J. Pritchard

After foetal death, survival of the placenta was investigated by Newton (1935) in the mouse, and growth of the placenta in the cat by Courrier & Gros (1936).

The normal rat placenta consists of decidua and foetal tissues. The foetal tissues are ectodermal giant cells and reticularis, both vascularized by the maternal vessels only, together with ectodermal labyrinth vascularized by both maternal blood and mesodermal allantoic vessels growing in from the embryo. The union between the mesodermal and ectodermal tissues occurs at the 11th day.

Foetal death was produced by four methods: direct foetal crush at laparotomy, double ovariectomy, injection of oestrone, and injection of follicle-stimulating hormone from mares' serum.

The results are shown in the accompanying table:

Date of interference	Foetal crush	Oestrone	F.S.H.	Ovariectomy
At 10th day before mesodermal ingrowth	Decidua normal. Trophoblast grows independently until 17th day	Acute necrosis of decidua and placenta	Decidual hypertrophy with ischaemic necrosis. Trophoblast grows independently	Decidual necrosis with complete destruction of embryo and trophoblast
At 11th day	—	—	—	Partial decidual necrosis. Death of foetus. Trophoblast grows
At 15th day	Placenta stable till full term. Ectoderm grows. Mesoderm survives	Normal growth	Normal growth	*Either* foetal survival with normal placenta *or* abortion with retention of placenta, partially separated from uterine wall

Killing at the 10th day yields a mesoderm-free trophoblast which grows from a diameter of 0·5 mm. to one of 5 mm. at the 16th day. This ectodermal trophoblast differentiates into its three zones with a normal type of evolution including well-developed glycogen accumulation. The mesoderm-free 'placenta' is destroyed on the 17th day by rupture of maternal vessels into the glycogen zone. If the foetus is killed after the mesoderm has grown into the placenta, the latter persists to full term, growth, however, being restricted to the trophoblast. The decidua either necroses or absorbs with ovariectomy, oestrone or F.S.H. injections. This decidual destruction impairs the growth of the rest of the placenta.

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The influence of pregnant-mares' serum on the pregnant rat.

By A. St G. Huggett and J. J. Pritchard

Abortion and resorption of foetuses following implantation of fresh anterior pituitary or injection of pregnancy urine have been described by Engle & Mermod (1928) in rats and mice, by Hill & Parkes (1932) in the rabbit, and by Coco (1942) in the rat. Alternatively pregnancy was prolonged. We have found similar effects in the rat following a single subcutaneous injection of F.S.H. derived from pregnant-mares' serum (Gestyl-Organon). The cause of foetal death, and the subsequent changes in the placenta have been investigated.

Foetal death is most easily produced by injection at the 10th day of pregnancy.

After the 12th day, the only effect of F.S.H. is to delay parturition. If retained after the 23rd day, foetuses die. (Full term is 21 days normally.)

50 i.u. is the minimum effective dose at the 10th day. This takes 3 days to produce its maximum effect. Ovarian weight also reaches its peak in 3 days. From 4 to 6 days after injection, induced follicles become luteinized.

Histology. Normally the allantoic circulation through the placenta is established on the 11th day. Lethal doses of F.S.H. given on the 10th day inhibit the penetration of allantoic blood vessels into the trophoblast.

The decidua basalis is abnormally thick owing to a failure of normal involution, remaining that of the date of death, is reduced in vascularity, and shows large areas of coagulative necrosis. In most cases the trophoblast survives, grows, and differentiates, in comparatively normal fashion, in spite of decidual necrosis, absence of allantoic mesoderm, and absence of foetus. In particular, the giant cells and glycogenic trophoblast are well developed. The labyrinthine trophoblast is partly destroyed by haemorrhage. It is probable that the decidual changes are the ultimate cause of foetal death after F.S.H.

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Effect of acceleration on cats, with and without water immersion.

By A. D. M. Greenfield

Forty cats, anaesthetized with chloralose, were centrifuged to produce accelerations up to 20 G in the head-tail axis measured at the heart. Continuous records of acceleration and of arterial, right auricular and intrapleural pressures were obtained. Without water immersion, arterial pressure showed an abrupt fall with onset of acceleration, reached zero at the head level at

3–4 G, and remained approximately constant during 1 min. runs. Right auricular pressures fell, but this was partly offset by the fall in intrapleural pressure. Following the run, the auricular pressure returned to its resting value, but the arterial pressure rose for 1–3 min. to a height greatly exceeding the resting value.

Immersion in water at body temperature to levels lower than 4 cm. below the cardiac apex made little difference to the response, but with the water level at the cardiac apex it required an acceleration of about 10 G to reduce the arterial pressure to zero at the head. After the first 10 sec. of the run, arterial pressure showed an increase of 60–80 mm. Hg, which was abolished by carotid sinus denervation. When this compensation had occurred, it required 15–20 G to reduce arterial pressure to zero at the head, corresponding to a pressure of about 300 mm. Hg at the heart. The right auricular pressure showed only a slight rise during the run. Following the run the arterial pressure rose above the resting value for 1–3 min. by an amount proportional to the compensation occurring during the run. This rise was also abolished by carotid sinus denervation.

Raising the water level above the cardiac apex gave only slight further improvement of the arterial pressure response, but the right auricular pressure showed a greater rise during the run.

Respiration was slowed and became shallow with occasional gasps in the non-immersed animals, but was well maintained in the water-immersed animals up to 16 G.

Following exposure to 15–20 G with water immersion for several half-minute runs, death frequently occurred, and subendocardial haemorrhages were observed in the left ventricle.

Part of the expenses of this research were defrayed by a Grant from the Governments Grants Committee of The Royal Society.

Physiological studies on animals subjected to positive *G*. By
H. H. Jasper and A. J. Cipriani. *From the Neurological Institute of McGill University*

Experiments were conducted on cats and monkeys (*M. mulatta*) with or without hydrostatic protection. A small centrifuge was used having a radius of 6 ft. and yielding a maximum of about 12 G. When possible, anaesthesia was avoided, but in some experiments light nembutal was given. Pressures were measured by means of a small photoelectric manometer which was practically isometric and not itself affected by G. Recording was by electrical methods and motion pictures.

In an unsuccessful search for an objective criterion of black-out, records of physiological interest were taken of brain waves, retinal potentials and the occipital cortical response to flashes of light. With the application of sufficient G for a long enough time, the E.E.G. showed an excitatory phase, followed by a train of delta activity leading to extinction of all rhythms. On recovery there was usually epileptiform activity followed by a gradual return to normal. Clinical attacks accompanied epileptiform activity. Retinal potentials showed a diminution in the amplitude of the B component which eventually disappeared. If the G was low the E.E.G. outlasted the B wave, but if the G was high and of rapid onset the brain waves were the first to disappear. The occipital cortical response disappeared shortly before the B wave of the retinogram.

Also: (1) Intracranial pressure decreased and became negative in a linear manner with the application of positive G. (2) Carotid arterial blood pressure and pulse pressure fell rapidly to zero with sufficient application of G. (3) Venous pressures in the femoral vein obeyed hydrostatic laws, but if measured below a valve showed a time lag in relation to measurements made above the valves. (4) Moving pictures of the cerebral vessels taken through a Forbes window under G showed a blanching of the cerebral surface, retention of blood in the larger vessels and slight movement of the brain, so long as the skull was intact. When the skull was punctured the brain sank under the application of G.

Hydrostatic protection tended to preserve the *status quo* to an extent which depended on the level of protection and the magnitude of the applied force. In fully protected animals subjected to high G for long and repeated periods the E.K.G. indicated right myocardial failure, and pathological examination revealed haemorrhage in the muscle of the right ventricle and the bases of the lungs.

This work was supported by a grant from the Associate Committee on Aviation Medical Research, the National Research Council of Canada.

Investigations on centrifugal force. By W. K. Stewart.

From the R.A.F. Physiological Laboratory

This problem has been studied in four general ways: (1) by experienced subjects in experimental aircraft, (2) by physiologists piloting various aircraft, e.g. Diringshofen, Davidson, (3) by mass study of pilots and their reactions, (4) in man-carrying centrifuges.

The major effects of centrifugal force on man result from the increased weight of his body components, especially the blood. Carotid blood pressure and cardiac output are lowered, with impairment of circulation and of central

nervous functions. Of these, vision suffers first owing to the retinal circulation being opposed by the intraocular pressure which, if artificially raised, lowers the *G* threshold for blacking-out. Total failure of vision may be preceded by a progressive rise of threshold and light sense may outlast visual acuity. In a large group of pilots, large unaccountable differences in threshold occur. The average difference between greying of vision and black-out is 0·7 *G*. Central circulatory failure results in brief unconsciousness (not necessarily preceded by black-out if *G* is excessive and suddenly applied) followed on recovery by marked confusion or disorientation.

Raised blood sugar, benzedrine or adrenal cortical hormone scarcely influence visual impairment, and since susceptibility to the cerebral effects of *G* is increased during flight by O_2 lack (if severe or reinforced by CO) the effects described are attributed to retinal and cerebral anoxia. Occlusion of the leg circulation raises and reactive hyperaemia lowers the black-out threshold to *G*. During the partial visual impairment of a prolonged manoeuvre, muscular effort (abdominal straining) may raise the blood pressure and restore normality. A pilot's resistance to the stress may depend on his general circulatory reactivity.

Duration is important. 20 *G* for 0·01 sec. or 12 *G* for 0·1 sec. causes no visual or neurological disturbance. (Tolerance of brief large forces probably depends on the structural strength of the body.) Several minutes of acceleration insufficient to impair vision cause only fatigue. In flight, the acceleration which affects vision usually reaches its maximum before reflex compensation (autonomic or somatic) can occur.

Increased weight may immobilize the trunk, but the limbs if supported are movable at right angles to the acceleration. Rapid rotation of the head (or a turret) at about 20°/sec. under high *G* may cause temporary disorientation, otherwise vestibular disturbances are uncommon in experienced pilots.

Description of a centrifuge and its use for studying the effects of centrifugal force on man. By W. R. FRANKS, W. K. KERR and B. ROSE. *R.C.A.F.* (*Toronto*)

In order to investigate the effects of centrifugal force on man, a centrifuge was constructed for the R.C.A.F. It is built into a circular concrete pit 12 ft. in depth and 31·5 ft. in diameter. To the central shaft which is supported above and below, a single, highly stressed horizontal arm, 8·5 ft. in length, is attached. The car which carries the subject and up to 200 lb. of apparatus has an inside diameter of 6 ft. 2 in. and is suspended from the distal end of the horizontal rotating arm. The centrifuge is stressed to support 15 times the weight exerted by a fully laden car at 10 *G*. (1 *G* equals the force due to the pull of gravity. Centrifugal force is measured in units of *G*.)

The suspension of the car allows it to assume at all times a position which is in the direction of the resultant of the forces acting upon it. The subject sits in a chair equipped with a dummy control stick, and rudder bars which can be adjusted for height and length. Within the car, the chair can be rotated around its transverse axis even when the centrifuge is in motion. Thus the centrifugal force can be exerted from head to foot (positive G), foot to head (negative G), transversely (transverse G) or in any intermediate direction. During a run, with the subject seated in the upright position, the radius is 11·5 ft. from the central rotating shaft to heart level and usually 13·8 ft. to foot level. The G exerted at foot level is therefore 20 % greater than the recorded G at heart level.

The centrifuge is driven by an electronically controlled 200 h.p. electric motor and is capable of developing 20 G in 3 sec. The magnitude of G and its duration for a given run is predetermined by an automatic cam mechanism. For a standard run, it takes 4·5 sec. to attain 1·5 G, and 5 sec. to attain any maximum desired G. When this has been maintained for 5 sec., the run is terminated by reversing the above procedure. This pattern can be varied by using appropriate cams. Runs are designated as follows. A '6 G run for 5 sec.' implies that 6 G was exerted at heart level for a duration of 5 sec.

The subject is in constant view of an observer who rides on a seat fixed to the central shaft. Electrocardiograms, electroencephalograms, ear opacity tracings and respiratory tracings are all recorded simultaneously with the visual and auditory signals given to the subject, his responses, signals indicating grey-out, black-out, loss of consciousness, the amount of G and the time in seconds, by ink-writing pens on moving paper. Ciné films and X-rays of the subject can be taken during operation.

This work was supported by grants from the Associate Committee on Aviation Medical Research, the National Research Council of Canada.

Some effects of centrifugal force on the cardio-vascular system in man. By W. R. Franks, W. K. Kerr and B. Rose. *R.C.A.F.* (*Toronto*)

By means of the centrifuge, the effects of increased positive G on the cardio-vascular system were studied on seventy-two subjects during 690 tests at 2–10 G. Kodachrome motion pictures showed, as increased G was applied, blanching of the face and distension of the superficial leg veins which persisted until a few seconds after the G began to diminish. The leg veins then reverted to normal, but the facial blanching was followed by flushing which lasted 10–20 sec.

The ear opacity (a qualitative measure of the blood content of the ear) began to decrease with the onset of increased G, reaching a minimum 4–6 sec. after G became constant. 0·5–3 sec. after the G began to decrease, the ear

opacity rapidly increased. The increase continued above the initial level, coincident with the facial flushing. The decrease in ear opacity was directly but not quantitatively related to the amount of G applied.

The heart rate increased rapidly with the onset of increased G, attaining a maximum of 120–190 beats/min., depending upon the amount of G and its duration. When the maximum G was maintained more than 10–20 sec., the maximum heart rate was relatively constant until the G was reduced. With the reduction of G in short runs there was a delay of 2–5 sec. before the heart rate suddenly fell to below its initial resting level. This bradycardia coincided with the flushing and increased ear opacity, and was frequently followed by a secondary rise in rate.

Electrocardiograms from chest electrodes over base and apex of the heart showed the following changes during increased G. The P.R. interval was shortened. The overall amplitude of the Q.R.S. complex decreased, usually with the main deflexion downward. The T wave flattened and sometimes disappeared. As the G was reduced, the P.R. interval and Q.R.S. complex reverted to their original form, but the T wave became greatly increased in amplitude and sometimes biphasic for 2–5 min. During this period sinus arrhythmia and, more rarely, ventricular extrasystoles appeared.

Anterior-posterior X-ray films of the chest (1 sec. exposure) taken during increased G showed a marked reduction in cardiac shadow as compared to that of control films.

The circulatory changes described in this paper could not be related to the level of G at which a subject would black-out or lose consciousness. However, the pooling of the blood in the lower extremities, reduction in cardiac shadow, facial blanching, decrease in ear opacity and associated changes in heart rate appear to be dependent variables and throw some light on the action of increased positive G on man.

This work was supported by grants from the Associate Committee on Aviation Medical Research, the National Research Council of Canada.

Some neurological signs and symptoms produced by centrifugal force in man. By W. R. Franks, W. K. Kerr and B. Rose. *R.C.A.F.* (*Toronto*)

The neurological effects of centrifugal force in man were studied in 542 subjects during 5544 test runs at 2–10 G in the centrifuge. As a measure of performance during exposure to centrifugal force, the reaction time for manual responses to visual and auditory stimuli was recorded for 7853 stimuli during 626 tests at 2–8 G on 35 subjects, but it was not significantly increased, except for visual stimuli immediately before black-out.

As a result of exposure to increased G, however, convulsions frequently occurred, usually after loss of consciousness. (52 % of 230 subjects had convulsions in 40 % of 591 tests producing unconsciousness.) They were usually slight, clonic seizures involving all or some of the extremities, face and trunk. Less commonly, severe generalized convulsions were observed. These varied greatly and sometimes included a brief tonic state with neck and trunk in extension, occasionally with arms extended in pronation and legs drawn up in flexion. Conjugate movements of head and eyes to one side were sometimes observed. Usually violent jerks of the extremities and trunk terminated the seizure in 2–5 sec. Finally, a small number of slight convulsions were noted in fully conscious subjects. Dreams were frequently experienced, usually in association with convulsions. Paresthesias, confused states, amnesia and more rarely, gustatory sensations were noted with black-out and loss of consciousness, either with or without convulsions. Incontinence was never observed.

The susceptibility to convulsions varied greatly and could not be correlated with any of the measured characteristics of resting electroencephalograms, which were normal for 51 subjects. Records of facial blanching and flushing, ear opacity and electrocardiograms showed that convulsions started during the recovery phase of the circulatory changes.

Electroencephalograms taken from bipolar leads over the motor area of the cortex, during increased G, showed that alpha waves were replaced by high frequency, low-amplitude waves, in fully conscious subjects. With deep black-out and onset of unconsciousness, progressively slower waves (8–2 per sec.) of higher amplitude (50–200 μV.) usually appeared and remained until shortly before consciousness was regained. This pattern was not altered by convulsions.

Considering the small difference in specific gravities of cerebrospinal fluid and brain tissue and their anatomical dispositions, it is unlikely that the neurological effects described in this paper are due to any mechanical action of increased positive G on the brain other than diminished cerebral circulation.

This work was supported by grants from the Associate Committee on Aviation Medical Research, National Research Council of Canada.

Some subjective effects of angular and centrifugal accelerations.

By S./Ldr. A. K. McIntyre. *R.A.A.F.* (*Sydney*)

Different combinations of linear and angular accelerations are encountered with different methods of producing high values of G. Such abnormal environments may produce complex, unfamiliar sensations, the study of which may throw further light on the mechanism of spatial orientation.

In the orthodox type of centrifuge a sequence of apparent changes in gravitational orientation is experienced by the subject who has no fixed visual horizon. With the onset of rotation, a sensation of upward and backward

movement is noticed, which is replaced during constant angular velocity by the general sensations of increased weight; during deceleration, a feeling is experienced of falling with forward rotation around a transverse axis. Vertical nystagmus (quick component downward) is observed at this stage and continues for some seconds after cessation of rotation. These effects are related to the magnitude of the angular acceleration around the axis of the centrifuge.

In the Australian accelerator, the magnitude of G may be varied without the production of associated angular accelerations. This is achieved by moving the subject radially away from or towards the axis during rotation of the machine at constant angular velocity. No sensations of rotation are experienced by the occupants, since the desired angular velocity is attained so slowly that appreciable stimulation of the semicircular canals is avoided.

Variations in radial acceleration in this centrifuge produce illusions of changes in bodily attitude. The resultant between gravity and centrifugal force is accepted as if it were simple gravitational pull, even if its direction is nearly horizontal. Thus a subject, although actually lying on his back, feels as if tilted into the upright position during the application of G, while the observer, seated upright and facing the axis of rotation, experiences the illusion of lying on his back throughout the period of rotation.

The angular momentum of the centrifuge may produce another striking illusion. If the head is tilted in any plane other than that of rotation, strong sensations are experienced of turning in a plane at right angles to the actual head movement, apparently because of precessional phenomena in the labyrinths. The occurrence of nystagmus (in the plane of subjective rotation) indicates that the semicircular canals are involved. This phenomenon may also be observed in spinning aircraft, and may lead to gross spatial disorientation in the absence of visual clues.

PROCEEDINGS

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The effect of desoxycorticosterone acetate on the chloride content of the sweat. By W. S. S. LADELL. *Medical Research Council, National Hospital, Queen Square, W.C.* 1

Daily intramuscular injections of 10–15 mg. of desoxycorticosterone acetate (DOCA) in man increase body weight and plasma volume, and reduce sodium and chloride excretion without changing serum sodium and chloride (Clinton & Thorn, 1943). Relatively larger doses in dogs (Clinton & Thorn *et al.* 1942), do increase serum sodium and chloride. Diminished urinary sodium and chloride may be the result of a direct action on the kidney tubules (Anderson, 1944). Is there a similar effect on the sweat glands? Serial collections of sweat were made from the arms of acclimatized subjects working in a hot room, before, during and after a course of injections of DOCA. Sweating rates were approximately the same for a given subject each day. Urinary chloride excretion was estimated; extra salt was given to ensure a minimum daily excretion of 10 g. Water was not restricted. 10 mg. of DOCA were given intramuscularly morning and evening. Sweat collected 12 hr. after the first injection was the same as on the control days; but sweat collected later contained less chloride, e.g. 0·45 % (as NaCl) compared with 0·65 % on the control days. No further change occurred though the subjects gained weight and became oedematous. The effect had passed off 72 hr. after the last injection. Total sweat loss of the subjects was measured, and assuming that arm

TABLE 1. Salt loss during sweating (Subject G.P.)

	Without DOCA	With DOCA	
No. of observations	4	3	
Rate of sweating (g./min.)	14·55	14·99	
Sweat loss g. of water	g. NaCl lost	g. NaCl lost	g. NaCl 'saved' by DOCA
250	1·356	0·850	0·506
500	2·566	1·696	0·870
750	4·509	2·781	1·728
1000	6·271	3·912	2·359
1250	7·742	5·109	2·633
1500	9·475	6·401	3·074
1750	11·768	7·918	3·850
2000	13·959	9·422	4·537
2250	16·447	11·098	5·349

sweat was a fair sample of the whole body sweat, the actual salt losses of the subjects each time were calculated.

Table 1 shows that about 30 % less salt was lost in the sweat when DOCA was administered.

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The effect of exercise on the renal blood flow in man. By J. A. Barclay, W. T. Cooke, R. A. Kenney and Marjorie E. Nutt. *The Department of Physiology, The University of Birmingham, and The Birmingham United Hospital*

Renal plasma flow (Diodone method) and inulin clearance (Barclay, Bray & Cooke, 1944) were determined on eight subjects before and after running 440 yards at maximum speed. In order to produce a diuresis, 800 c.c. of H_2O were drunk before taking exercise (in one case 2400 c.c. were required). The effect of exercise on the diuresis was noted.

Renal plasma flow before exercise ranged from 540 to 940 c.c./min. (average 703 c.c./min.). In all subjects the plasma flow fell as a result of exercise. The percentage decrease ranged from 18 to 54 % of the initial flow. The flow remained below the pre-exercise level for periods ranging from 10 to 40 min. after the exercise.

It is remarkable that the subject who had the lowest plasma flow (540 c.c./min.) showed the greatest decrease as a result of exercise, flow decreasing by 54 % to 249 c.c./min. In spite of this great decrease in renal plasma flow there was only a slight inhibition of diuresis. In one subject there was an increase in urine flow from 4·0 c.c./min. before exercise to 9·4 c.c./min. in the exercise period, in spite of a 40 % decrease in renal plasma flow.

Inulin clearance fell as a result of exercise in six cases, though in one of these the fall was very slight. In one case, although the renal plasma flow fell from 555 to 368 c.c./min., there was no fall in inulin clearance.

In four subjects exercise produced an inhibition of diuresis, in four there was no inhibition.

In a further two subjects the period of exercise was increased to 15 min., a bicycle ergometer being used. In both of these subjects there was a fall in plasma flow and in clearances, which continued throughout the exercise. In one of them there was a marked inhibition of diuresis and no evidence of restoration of urine volume during the next few hours. In the other subject there was a slight inhibition during the exercise period, but diuresis was re-established as soon as exercise ceased.

Renal blood flow decreases as a result of taking exercise, but this decreased blood flow cannot be responsible for the inhibition of diuresis which may be produced by the exercise, nor is the inulin clearance decreased in every case. It would seem, therefore, that the volume of urine produced and its composition is in a large measure independent of the blood flow through the kidney. The resting kidney has such a wide 'safety margin' that the renal blood flow may be cut down by half without any appreciable effect on the volume of the urine produced.

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Hyperpnoea of effort. By J. N. Mills. *Department of Physiology, University of Oxford*

The hyperpnoea at the onset of exercise was ascribed by Krogh & Lindhard (1913) to irradiation of impulses from the cerebral cortex. The need to invoke a cortical mechanism has, however, diminished since the recognition of various reflex and other nervous mechanisms (Comroe, 1944). Bedford, Vernon & Warner (1933) have shown that static effort often induces a hyperpnoea out of proportion to the metabolic increase, which they ascribe to the onset of pain and fatigue. It has been found that a brief period of static effort with one hand, which gives rise to neither pain nor fatigue, induces in some subjects a similar hyperpnoea, which would seem to be of cortical origin.

Respiratory tracings were obtained by the usual Krogh spirometer technique from subjects who, in the middle of a period of rest, squeezed an inflated rubber bag as powerfully as possible with one fist, maintaining the effort for a period of 10 sec. From such a tracing it is possible to calculate the resting ventilation and oxygen consumption, and the extra ventilation and oxygen consumption induced by the effort.

Forty-five observations have been made upon sixteen different subjects. In almost every tracing an inspiratory hypertonia accompanied the effort. In a few subjects respiration returned to normal almost immediately afterwards, but in the majority there was some continued hyperpnoea. The total volume of air inspired during the period of respiratory disturbance exceeded the resting value for an equal period of time by from zero to 34 l., or up to 455 % of the volume inspired per minute at rest. The excess oxygen consumption varied from an indetectable amount to 213 c.c., or up to 70 % of 1 min. resting consumption. Eight of the subjects ventilated more than could be accounted for by their metabolic increase. A respiratory acceleration in anticipation of exercise was often seen, as in some of the tracings of Krogh & Lindhard. The various non-chemical factors in exercise hyperpnoea have been discussed

by Comroe (1944), but none seems relevant to the present experiments, which appear to involve a cortical mechanism.

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The effects of diets deficient in tryptophan, lysine, and calorific value on the liver cytoplasm of rats. By H. W. Kosterlitz and Rosa M. Campbell. *Department of Physiology, Marischal College, Aberdeen*

When rats were transferred from the stock diet to a protein-free diet, 15 % of their original liver cytoplasm was lost on the 1st day, 8 % on the 2nd day, 3 % during the 3rd and 4th days, while during the next 3½ weeks the daily mean loss was 0·8 %. Thus, of the 'labile liver cytoplasm' (Kosterlitz, 1944) approximately 60 % was lost in 1 day, 90 % in 2 days, and 100 % in 4 days. Nothing can yet be said about the possible chemical differences between the labile and the remaining liver cytoplasm.

When rats were placed on a tryptophan-deficient diet containing 17·3 % zein plus 1·28 % *l*(+)lysine hydrochloride, their liver cytoplasm, as measured by the non-lipin non-glycogen liver solids, decreased more slowly than on a protein-free diet, viz. by 7 % in 2 days, 16 % in 4 days, and 24 % in 7 days. On a lysine-deficient diet containing 18·3 % zein plus 0·34 % *l*-tryptophan only 9 % liver cytoplasm was lost in 7 days; there was no loss in rats fed for 7 days on a diet containing 17 % zein plus 1·28 % *l*(+)lysine hydrochloride plus 0·34 % *l*-tryptophan. These results agree well with the known facts regarding the relative indispensability of the two amino acids for the adult animal.

Feeding of 3·75 g./100 g. body weight daily of a 20 % casein diet caused losses in liver cytoplasm of 14, 15 and 27 % in 2, 4 and 7 days respectively. In contrast to this, rats fed with 7·1 g./100 g. body weight daily of a 10 % casein diet lost only 10 % of their liver cytoplasm in 7 days. Feeding with 40 and 60 % casein diets at a daily rate of 3·75 g./100 g. body weight for 7 days caused decreases in liver cytoplasm of 16 and 11 % respectively, while rats fed at a daily rate of 7·0 g./100 g. body weight lost no liver cytoplasm when the diet contained 20 % casein and gained 4 % liver cytoplasm when the diet contained 30 % casein. It is likely that the rapid loss of liver cytoplasm in rats fed on the low-calorie diet containing 20 % casein is due to the utilization of protein for energy; it is, however, of interest to note that this loss is considerably reduced by raising the casein content to 40 or 60 %.

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Factors influencing the formation of acetylcholine in cell-free extracts from brain. By W. Feldberg and T. Mann. *Physiological Laboratory and the Molteno Institute, Cambridge*

Both homogenized brain tissue (Nachmansohn & Machado, 1943; Nachmansohn, John & Waelsch, 1943) and cell-free extracts prepared from acetone-dried brain (Feldberg & Mann, 1944) contain an enzyme system which synthesizes acetylcholine provided that adenosinetriphosphate (ATP) is added. The extract from 1 g. acetone powder (rat) can form in the presence of ATP up to 400 μg. acetylcholine in 1 hr. at 37° C. In order, however, to achieve such a high yield of acetylcholine large amounts of ATP had to be added (4–10 mg. Pyro-P) which exceed by far the concentration of ATP normally present in brain.

Recently, however, we succeeded in showing that ATP is not an irreplaceable component of the synthesizing system. An equally large formation of acetylcholine could be obtained both aerobically and anaerobically if, instead of ATP, citrate and boiled juice from brain were added. The boiled juice was fully active only in the fresh state. The activator present in it is not identical with either ATP or choline. Whereas the activating effect of ATP on the formation of acetylcholine is reversed by glucose, owing to the esterification of the labile phosphate groups of ATP, the synthesis of acetylcholine in the presence of citrate and boiled juice is not influenced by glucose.

If all three activators, ATP, citrate and boiled juice, were added together to the brain extract, the rate of formation of acetylcholine rose to 1200 μg. (rat) and 1800 μg. (guinea-pig) per g. acetone powder, 1 hr., 37° C. This is the highest rate hitherto observed. Other corresponding average figures for rat and guinea-pig respectively are 300 and 700 μg./g. with ATP alone, 700 and 900 with ATP and boiled juice and 1000 and 1200 with ATP and citrate.

Citric acid could not be replaced by succinic, fumaric or tartaric acids. Malonic, glutamic and aconitic acids had only a slight activating effect. Malonic acid did not inhibit the action of citric acid.

On dialysis the brain extract lost the ability to form acetylcholine. Its activity, however, could be restored by citrate and boiled juice added together, or by citrate and ATP added together, but not by citrate alone nor by the boiled juice alone.

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The formation of lipoprotein complexes at the oil/water interface and in solution. By J. J. Elkes, A. C. Frazer and J. H. Schulman. *Pharmacology Department, Birmingham, and the Colloid Science Department, Cambridge*

The adsorption of proteins to charged oil/water interfaces is a *p*H-conditioned and reversible phenomenon (Adair, Elkes, Frazer, Schulman & Stewart, 1944). The relationship between the soap concentration used to stabilize the emulsion with a constant interfacial area and the flocculation phenomenon has been further studied, and compared with the effect of varying concentrations of soap solutions on protein in the absence of an oil/water interface. The protein used was human haemoglobin and the emulsions were olive oil emulsions of 0·5 μ droplet diameter stabilized with 0·001–2·0 % sodium hexadecyl sulphate. All mixtures were buffered with citrate NaOH buffer to *p*H 5·1.

With increasing soap concentrations more protein was required to produce flocculation of the emulsion, but it always occurred at a protein/soap ratio of 2/1 by weight or less. Protein/soap ratios above 2/1 did not interfere with flocculation. With soap solution alone, protein precipitation only occurs when the protein/soap ratio lies between 2·5/1 and 5/1 and not above or below this ratio, which is in accord with the findings of Putnam & Neurath (1944).

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PROCEEDINGS

OF THE

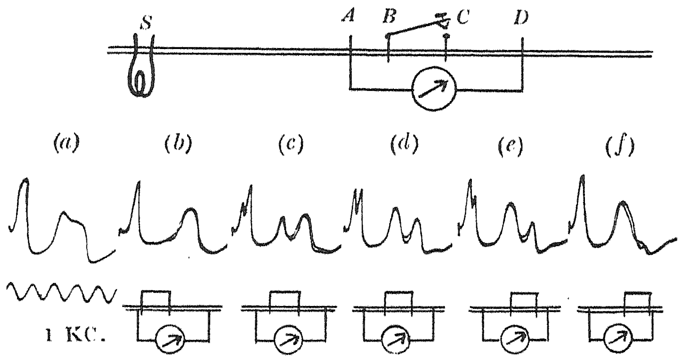
PHYSIOLOGICAL SOCIETY

2 *June* 1945

Resistance artifacts in action potential measurements.

By W. A. H. RUSHTON. *Physiological Laboratory, Cambridge*

From an application of Ohm's Law to current distribution about a cable one may deduce the following theorem which is easy to state and has a rather wide application. The principal assumptions are: (*a*) that in the external medium about a nerve fibre, radial lines may be considered equipotential, and (*b*) that the resistance per unit length of the external medium is small compared with



that of the axis cylinder. Uniformity of the nerve is *not* assumed, neither constant conduction velocity, nor similarity of action potential from point to point, nor uniformity of external or internal resistance.

THEOREM. *The action potential recorded between points A and B on the surface of a nerve depends only upon the properties of the stretch AB and is quite unaffected by properties elsewhere.*

It has long been recognized (see references) that if between the recording leads *AD* there is a region *BC* of low resistance, e.g. a drop of fluid, a metallic bridge, etc., a change occurs in the recorded action potential.

Our theorem gives precise expectation of the effect of short-circuiting *BC*, and the records show some experimental results in the case of giant nerve fibres from the earthworm.

(*a*) Normal action potential from *AD*. First wave from median, second from the two lateral giant fibres in unison. Time: 1 kc./sec.

(*b*) to (*f*) Action potential with short-circuit *BC* in various positions. The expectations from the theory are computed electrically and superposed by a second photographic exposure. The correspondence is encouraging.

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The fixation of globulin by guinea-pigs given a sensitizing injection of globulin followed by albumin.

By L. B. Winter. *Department of Physiology, University of Sheffield*

It has been shown (Winter, 1944) that, after a sensitizing injection of globulin has been given to guinea-pigs, followed 19 days later by albumin, there is no appearance of sensitivity to albumin in the uteri after a further interval of 31 days. When the amount of globulin required to desensitize these guinea-pigs (termed G.-A. animals) *in vivo*, was compared with that needed by animals given a sensitizing injection of globulin alone, it was found that the latter required more than ten times the amount of antigen. The G.-A. animals were also more susceptible to shock than were globulin animals examined soon after they became sensitive. It was suggested, therefore, that the antibodies formed in the two types of animal were not identical.

Recent work (Winter, 1945) has shown that there is a significant increase in the susceptibility to shock of globulin-sensitized animals between 18 and 50 days, and also that different protein preparations vary in antigenic activity. For these reasons, a more accurate comparison has been made between globulin-sensitized and G.-A. animals. Virgin guinea-pigs were used, weighing about 200 g. at the time of sensitization. All the animals were sensitized by globulin; half received an injection of albumin 19 days later. About 50 days after the first sensitizing injection, the guinea-pigs were given injections, by the portal route, of the same preparation of globulin. The desired amount of antigen was given in three or four injections, and symptoms of shock occurred in only one animal. The first uterine horns of 12 G.-A. animals were tested with 0·5 mg. albumin, then with 0·1 c.c. of serum: there was a response to serum in each case, none to albumin. Twelve sensitive globulin animals were used; the uteri were tested with serum. The border-line dose of antigen needed to desensitize the globulin animals *in vivo* was 1·0 mg./100 g. Since the same sample of globulin was used as in the previous work (Winter, 1945), comparison with Table 3 of that paper shows that globulin-sensitized guinea-pigs require the same amount of globulin for desensitization at 18 and 50 days. The corresponding amount of globulin for desensitization of the present series of G.-A. animals was 0·17 mg./100 g. It is doubtful whether the smaller difference in

the fixation of globulin, unsupported by other evidence, is sufficient to decide whether albumin plays some part in antibody formation in G.-A. animals, or whether these animals are identical with those sensitized by globulin alone.

The expense of this work was in part defrayed by the Government Grants Committee of the Roya Society.

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The uterine response to albumin and globulin in guinea-pigs sensitized by these proteins. By L. B. WINTER. *Department of Physiology, University of Sheffield*

Using horse-serum proteins, it was shown (Winter, 1944) that in the four series of animals sensitized by (*a*) serum, (*b*) synthetic serum, (*c*) globulin followed by albumin (G.-A. animals), and (*d*) albumin followed by globulin (A.-G. animals), the uteri of the last series only were responsive to albumin *in vitro*. The sensitivity of the uteri of the A.-G. animals to albumin was, however, discharged by an injection of globulin into the animal, and it was suggested that these uteri would be responsive *in vitro* both to globulin and to albumin, but that the sensitivity to both proteins would be discharged by either of them. This has been found to occur, with few exceptions.

Twenty-three virgin guinea-pigs, weighing about 200 g., were used. The method of sensitization was as described (Winter, 1944) for A.-G. animals, and the proteins were prepared in the same way. Under ether anaesthesia, two portions of a single cornu were removed, and each was placed in a separate bath. To one bath globulin was added, followed later by globulin, and then, after changing the Ringer's solution, by albumin. Albumin was introduced into the other bath, followed by albumin, then by globulin. No response was ever obtained from the second introduction of the antigen first used. In twelve experiments 1 mg. of protein was introduced on each occasion into the bath, in the remainder 0·5 mg. The smaller amount was more than sufficient to cause a maximal contraction, and there appeared to be no advantage in using the larger quantity, nor was there any difference in the results. It was shown in the previous paper that when a response could not be elicited by 1 mg. of protein in the bath, 5 mg. had no effect.

Of the twenty-three guinea-pigs used, the uteri of nineteen were sensitive both to globulin and to albumin. Two were sensitive to albumin alone, one to globulin alone, and one to neither protein. Of the nineteen uteri which had dual sensitivity, only two showed a response to albumin after globulin; one

responded to globulin after albumin. With these exceptions, we may conclude that when sensitivity to albumin and globulin is present in the uterus, either antigen will discharge the sensitivity to both proteins.

The expense of purchase and maintenance of the animals was defrayed by the Government Grants Committee of the Royal Society.

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The water content of embryonic tissue in the sheep. By A. CARLYLE (introduced by J. BARCROFT) (*from the Cambridge Unit of Animal Physiology, A.R.C.*)

It is known that in general the percentage of dry matter in the foetus increases as foetal life proceeds, and there is fragmentary information about individual tissues in one species or another (Needham, *Chemical Embryology*, p. 888) but, with the exception of figures for the sheep at one age (Davidson & Waymouth, 1944), no body of information exists sufficient to form the basis of an integration of the oxygen consumption of the complete foetus from estimations of the Q_{O_2} of the slices of its individual tissue. With this end in view the following observations have been obtained:

TABLE I. Showing the dry weight of tissues of the sheep foetus, at different ages, and of one lamb, expressed as a percentage of the fresh weight

	Age of foetus (in days)									
	72	78	103	112	120–2	126	135–6	142–4	Lamb 6 days	Adult
Skin	9·0	10·6	12·8	—	16·1	18·5	24·2	21·9	22·3	29·6
Bone	27·1	33·9	49·0	—	42·0	34·0	46·6	45·4	56	67
Cartilage	—	—	21·4	—	29	—	20·6	22·5	22·0	35·0
Muscle	9·6	10·1	11·4	—	17	18·5	17·8	19·2	20·7	26·6
Liver	17·6	19·4	19·3	—	19·2	18·9	21·0	26·1	23·0	30·0
Gut	12·2	12·2	—	14·8	14·5	15·1	13·2	14·1	17·1	22·3
Lung	9·7	11·6	11·1	—	11·1	9·3	11·4	14·5	21·5	21·2
Brain	10·6	9·5	—	10·4	—	—	13·2	15·6	15·2	24·3
Heart	12·5	—	—	15·3	—	—	—	12·2	19·0	18·3
Kidney	12·5	11·9	—	8·1	—	—	—	11·4	18·9	21·9
Blood	—	—	—	15·8	16·6	18·9	16·6	17·1	21·1	19·7

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The effect of juxtaposition of different colours. By F. W. EDRIDGE-GREEN (introduced by J. H. GADDUM)

When two different colours are placed side by side, one of the colours appears as if it had been mixed with the other. For instance, yellow against blue appears whitish or white, pure green against blue, appears blue-green. In ordinary circumstances there is no appearance of simultaneous contrast.

Most of the examples are by Mr P. Warburton, an artist.

PROCEEDINGS

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21 *July* 1945

The effect of induced auricular fibrillation on the work capacity of the cat heart. By A. MORGAN JONES and W. SCHLAPP. *Department of Physiology, University of Manchester*

Using a modified Starling heart-lung preparation, auricular fibrillation was induced by electrical stimulation through the auricles at a rate too rapid to permit a response to individual stimuli (50/sec.). Arterial and left auricular pressures were measured directly and the heart output was recorded continuously by a stromuhr. After the stimulus ceases the arrhythmia may continue for a few seconds without significant change of auricular pressure, arterial blood pressure or heart output. Electrocardiograms taken during this period show that the arrhythmia fulfils the criteria used for the recognition of human auricular fibrillation. The auricular and ventricular rates are approximately double the corresponding ones in man.

By plotting curves relating the mean auricular pressure to the effective rate of work of the heart (mean arterial pressure × heart output) it is possible to compare the work capacity of the heart during normal rhythm with that during fibrillation. It has been found that fibrillation reduces the work capacity of the heart by approximately one-third (e.g. 554 to 370 g.metres/min.) (Fig. 1).

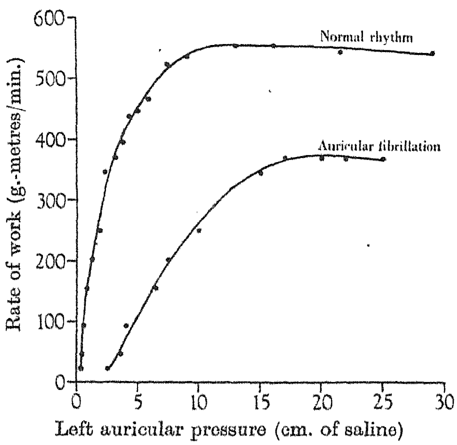


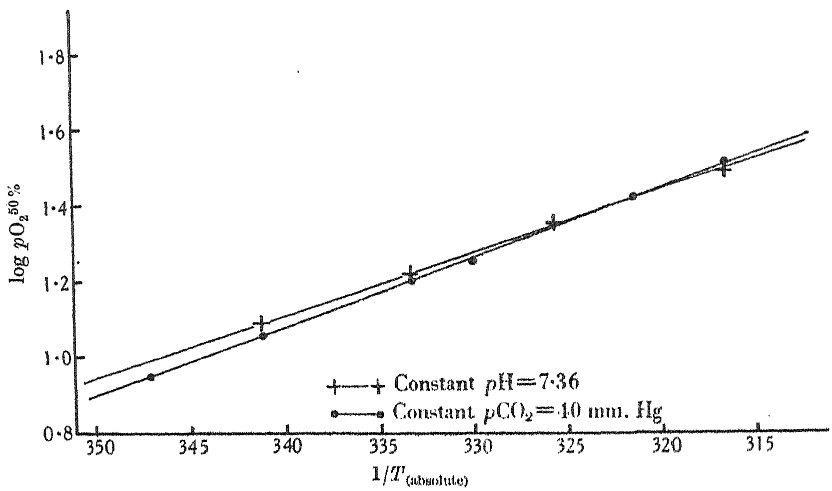
Fig. 1

When fibrillation is induced at work rates below the maximum fibrillating work rate, the subsequent events can be divided into three periods. At the onset of the arrhythmia the heart output rapidly falls, and then more slowly rises to the original level; this is accompanied by a rise of auricular pressure. This initial period we have called *adaptation*. When the heart output has returned to the original amount, the auricular pressure stops rising and a period of *stabilized fibrillation* begins. This continues until normal rhythm is restored, when a period of *recovery* ensues; during this the heart output rises, and remains above the original amount until the deficit acquired during adaptation has been quantitatively made up.

When the rate of work before fibrillation exceeds the maximum fibrillating work rate, the original output is not maintained at any time during fibrillation. A stabilized state is never reached for, so long as the arrhythmia continues, the auricular pressure progressively increases and the heart dilates; ultimately failure ensues. It has been possible to prevent this only by reducing the work of the heart.

The effect of temperature on the oxygen dissociation curves of human blood. By E. Neil. *Department of Physiology, School of Medicine, Leeds*

The comparison of oxygen dissociation curves determined at different temperatures requires a standardization of either CO_2 pressure or of pH. Thus a standard pCO_2 of 40 mm. Hg may be employed or the pH of the blood may



be kept constant—preferably at a normal value of 7·34 ± 0·03. Stadie & Martin (1924) first pointed out that the comparison of these curves should be made under isohydric conditions. But if pCO_2 is kept constant at 40 mm. the blood pH varies at different temperatures, being more alkaline at temperatures below 38° C. and more acid at higher temperatures when compared with its normal value at 38° C. Curves calculated by Brown & Hill (1922–3) do not allow for this.

Experiments have been performed on the author's blood to investigate the effects of temperature on blood-oxygen dissociation curves at constant pH or at constant pCO_2.

pH was determined using Hawkins' method (1923) *or Hastings & Sendroy's method* (1924) *checked with the glass electrode. Blood oxygen was determined*

with the Van Slyke manometric apparatus, and gas mixtures were analysed with the Haldane gas apparatus. For each temperature, $pCO_2 : 1/K$ lines and $\log 1/K : pH$ lines were constructed. As $\log 1/K = n \log pO_2$ at 50% saturation ($n = 2\cdot3$), the relationship between pO_2 at 50% saturation and temperature can be plotted as in the accompanying figure. This relationship is depicted for $pCO_2 = 40$ mm. Hg and for $pH = 7\cdot36$. The slope of the line plotted at constant pCO_2 is greater than that at constant pH which confirms the deductions of Stadie & Martin (1924).

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Rhythmical fluctuations of the arterial blood pressure. By

A. Schweitzer. *Physiology Department, School of Medicine, Leeds*, 2

A survey of the descriptions given in popular text-books of physiology of the rhythmical variations seen in arterial blood-pressure records shows a surprising degree of terminological confusion, especially with regard to the influence of the respiratory cycle on arterial blood pressure. The so-called Traube-Hering waves of blood pressure are in some books related to respiratory activity; others refer to them as due to periodic variations in vasomotor tone, independent of respiration. This confusion appears evident in M. Foster's text-book (1883) and was certainly perpetuated by an account of Halliburton (1920) who attributes certain observations made by Sigmund Mayer (1876) to the group of respiratory modifications of blood pressure.

The original observations of Traube (1865) with regard to an irradiation of impulses from the respiratory centre to the vasomotor centre were confirmed by Hering (1869) and many others (cf. lit. in Schweitzer (1937)). Hering writes: 'We have adequately proved that the vascular system shows respiratory fluctuations which are associated with the respiratory movements and which, as the latter, originate rhythmically from the so-called respiratory centre.' The following classification of the main rhythmical fluctuations of the arterial blood pressure is suggested:

(1) Fluctuations due to cardiac activity.

(2) Fluctuations due to respiratory activity: (*a*) Mechanical effects of the respiratory pump mechanism. (*b*) Traube-Hering waves, due to irradiation of impulses from the respiratory to the vasomotor centre and synchronous with respiration.

(3) Fluctuations of blood pressure due to rhythmical variations in vasomotor centre tone which are independent of respiration and always much slower in rate (Sigmund Mayer waves).

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Adenosine triphosphate and renal blood flow. By C. A. Keele and D. Slome. *Departments of Pharmacology and Physiology, Middlesex Hospital Medical School, London*

The actions of the sodium and magnesium salts of adenosine triphosphate (A.T.P.) on blood pressure (B.P.) and renal blood flow (R.B.F.) have been studied in chloralosed cats. R.B.F. was measured by the vena cava shunt method of Barcroft & Brodie (1904) or the ovarian vein shunt method of Keele & Slome (1943).

ROUTES OF ADMINISTRATION

(1) *Rapid intravenous injection.* Na A.T.P. was given in doses ≡ 2–5 mg. of Ba A.T.P. (the parent compound from which Na A.T.P. and Mg A.T.P. were prepared) and Mg A.T.P. in doses ≡ 1·25–2·5 mg. Ba A.T.P.

(2) *Intravenous infusion.* Na A.T.P. and Mg A.T.P. were given in doses ≡ 72–116 mg. Ba A.T.P. during periods lasting $1\frac{1}{2}$–13 min.

(3) *Intramuscular injection.* Doses ranged from Na A.T.P. ≡ 50 mg. Ba A.T.P. to Mg A.T.P. ≡ 105 mg. Ba A.T.P.

With both Na A.T.P. and Mg A.T.P. and by all these routes of administration the B.P. was reduced and often the heart rate was slowed. In most cases the R.B.F. was also reduced, but apart from those experiments where the fall in B.P. was rapid and considerable the percentage reduction in R.B.F. was less than the percentage reduction in B.P., and in some cases the R.B.F. actually increased to exceed the initial value, when the B.P. was markedly lowered. This latter finding indicates renal vasodilatation.

It has been suggested by Green (1943) that A.T.P. may be liberated from damaged muscle in sufficient quantities to produce signs of shock, and that following crushing injuries to the limbs it might damage the kidney. We have previously observed (Keele & Slome, 1945) that following the release of limb ischaemia of 4–5 hr. duration in cats, the percentage reduction in R.B.F. is greater than the percentage reduction in B.P. This suggests that the renal

blood vessels were constricted, so it is most improbable that A.T.P., a renal vasodilator, is responsible for the reduced R.B.F. seen after release of limb ischaemia.

We are indebted to the Medical Research Council for a grant to Miss M. H. D. Chennells for technical assistance.

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A comparison of the effects on the pancreas of pancreozymin and of vagal nerve stimulation.

By A. A. HARPER and I. F. S. MACKAY. *Departments of Physiology, Manchester University and Sheffield University*

Harper & Raper (1943) have described the existence of a substance in extracts of the small intestine which caused an increase in the secretion of enzymes by the pancreas. They have called the substance 'pancreozymin', and suggest that it may be the hormone responsible for the secretion of pancreatic enzymes. They demonstrated the activity of the hormone by examining its effect on enzyme output. These observations have been extended to correlate them with cytological changes in the pancreas, and with the effects of vagal nerve stimulation upon the pancreas.

From starved cats a piece of pancreas was removed and stained by Bensley's method. Pancreatic juice was collected under secretin stimulation. In addition to the secretin stimulation the enzyme output was increased by (*a*) stimulation of the dorsal vagus trunk in the thorax, or (*b*) the intravenous administration of pancreozymin for periods of up to $3\frac{3}{4}$ hr. A second piece of pancreas was removed for histological examination at the end of the period of stimulation.

RESULTS

There was a good deal of variation in the enzyme granule content of the pancreas in starved animals. In control experiments in which the pancreas was not stimulated, or secretin only was administered, there was no significant change in the amount of enzyme granules in the pancreas. After stimulation of the dorsal vagus trunk or the administration of pancreozymin there was a marked decrease in the enzyme granules, but never a complete exhaustion of the gland.

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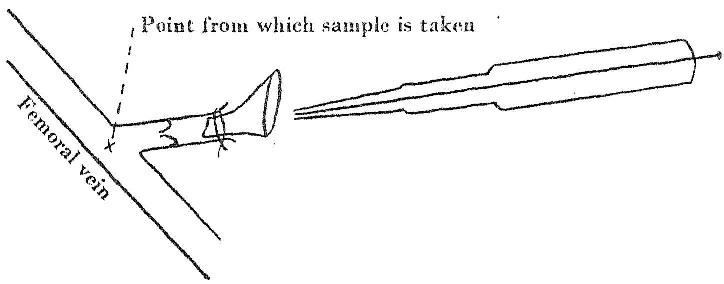
A method for obtaining uncontaminated venous blood for coagulation tests. By S. Brandon Stoker (introduced by D. Burns)

The cannula and pipette were designed and used to obtain venous blood from dogs, for the measurement of coagulation time.

The blood is obtained directly from the lumen of an undamaged vein, through one of its branches, into the pipette.



The branch vein and its guarding valve are washed by normal saline through the cannula, before and after each sample is taken.

The blood pipette is waxed, and sealed with a glass stylet. The tip of the pipette is introduced into the main vein through the cannula and aperture of the valve. By means of a twisting movement the seal is broken and the stylet removed when blood flows into the pipette.

Using this method it has been possible to obtain up to fifty samples in an hour.

A modification of the McLean-Hastings method for the biological assay of calcium ions. By D. H. Miller and E. Neil. *Department of Physiology, School of Medicine, Leeds*

McLean & Hastings (1934) were the first to assay calcium ions biologically, using a frog's heart perfused through a Straub cannula. The alterations in the amplitude of the recorded contractions caused by the addition of serum or other biological fluids were compared with those resulting from the addition of calcium solutions of known ionic strength.

In our hands the Hartung (1911) frog-heart preparation as modified by Clark (1912) has proved more satisfactory for this assay. The output of the heart is under observation and can be controlled, and the method of perfusion ensures a more adequate oxygenation. It is an advantage to add about 0·05 ml. of serum to 1·5 ml. of the circulating fluid, because the heart perfused

with only saline solutions becomes hypodynamic after some hours (Clark, 1913–14), and, in these circumstances, serum added for assay purposes will produce an augmentation of the beat, irrespective of its calcium content. It has also been found that the addition of creatine, to give a concentration of 0·2% to the solutions used by McLean & Hastings, results in stronger contractions. Our experience, unlike that of McLean & Hastings, is that the continuous passage of CO_2 through the perfusing fluid results in a better maintenance of the beat, the $CO_2/NaHCO_3$ system being adjusted to maintain a constant *p*H of 7·40 throughout perfusion.

In other respects the methods employed are those of McLean & Hastings. The results obtained by use of this preparation confirm the findings of these authors that the serum level of calcium ions is 4·25–5·30 mgm. per 100 ml.

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experimental procedures. A precipitate which settled on standing proved to be very rich in calcium.

The kidneys of all the animals were investigated histologically by Prof. T. F. Hewer. No abnormality was found.

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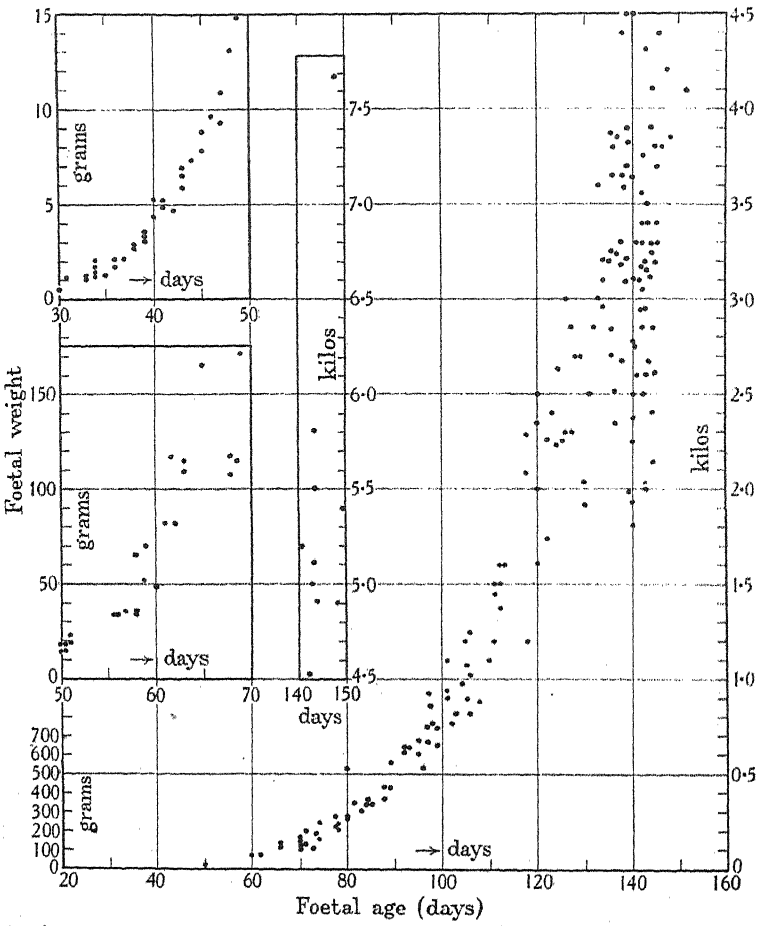
The range of weights of foetal sheep at various ages. By Joseph Barcroft. *A.R.C. Unit of Animal Physiology, Cambridge*



The figure shows the weights of sheep foetuses which have been subjects of operation at Cambridge. They were the offspring of Welsh ewes; the ram,

however, is unspecified. The weight at term may vary from 7·6 to 1·8 kg. This great diversity only appears after 130 days, nor is it shown on the crown-to-rump length (i.e. bone). It is suggested that some foetuses falter in the growth rate of some at least of their soft parts.

The composition of sheep foetuses. By L. R. WALLACE. *School of Agriculture, Cambridge*

Only very limited data have been published on the composition of sheep foetuses, and since there are wide differences, which may be of interest to those studying various aspects of foetal physiology, between the rate and order of development of component parts, a brief summary of the data from a small series is here presented.

Age of foetus (days) ...	56	84	112	140	56	84	112
No. of foetuses ...	3	6	3	3	3	6	3
	Mean weight of part g.				As % of weight of part at 140 days		
Thyroids	0·03	0·16	0·44	1·40	2·14	11·4	31·4
Neck thymus	0·04	1·50	10·54	32·12	0·12	4·7	32·8
Heart thymus	0·02	0·43	3·45	16·62	0·12	2·6	20·8
Heart	0·41	3·58	13·59	45·94	0·87	7·8	29·6
Lungs	1·02	20·54	71·80	171·25	0·60	12·0	41·9
Trachea	0·12	1·27	5·76	15·97	0·75	8·0	36·1
Diaphragm	0·16	2·98	9·24	28·58	0·56	10·4	32·3
Oesophagus	0·07	0·93	3·00	7·89	0·87	11·8	38·0
Rumen	0·13	1·27	4·84	13·37	0·97	9·5	36·2
Reticulum	0·04	0·45	1·77	5·12	0·78	8·8	34·6
Omasum	0·07	0·50	1·97	4·32	1·62	11·6	45·6
Abomasum	0·05	0·82	4·58	28·78	0·17	2·9	15·9
Small intestine		3·01	11·35	62·59	—	4·8	18·1
Caecum	0·69*	0·04	0·31	1·56	—	2·6	19·9
Large intestine and rectum		0·92	4·38	19·48	—	4·7	22·5
Gall bladder	5·32	0·08	0·14	1·14	2·2	7·0	12·3
Liver		41·25	123·27	244·80		16·9	50·4
Spleen	0·02	0·76	4·47	13·67	0·15	5·6	32·7
Pancreas	0·08	0·67	2·33	6·16	1·29	10·9	37·8
Bladder	0·03	0·56	2·12	4·58	0·66	12·2	46·3
Kidneys	0·46	4·60	13·57	36·03	1·28	12·8	37·7
Skin	4·34	47·52	296·23†	918·16†	0·47	5·2	32·3†
Flesh	17·66	223·96	807·09	2188·9	0·81	10·2	36·9
Skeleton	6·12	56·17	255·88	907·9	0·67	6·2	28·2
Brain	1·97	11·56	31·12	61·00	3·23	19·0	51·0
Foetus	45	501	1952	5866	0·77	8·54	33·3

* With contents. † With wool.

During an investigation on the manner in which foetal composition is affected by the level of nutrition of the ewe, a normal age series was obtained from thirteen Border-Leicester × Cheviot ewes, mated to the same Suffolk ram, fed a liberal standard ration, and killed at 28-day intervals from service. The foetuses were dissected fresh.

The weights of some of the major parts are tabulated above. It is apparent that organs such as the kidney, liver, lungs, bladder and brain make a larger

proportion of their foetal growth during the early stages than do parts such as the spleen, thymus, intestines and skeleton. The differential nature of growth is clearly evident.

The weights of certain tissues of the sheep foetus during gestation, relative to the total body weight. By A. Carlyle. *A.R.C. Unit of Animal Physiology, Cambridge*

For the purpose of integrating the oxygen consumption of the sheep foetus from estimations of the Q_{O_2}'s of slices of the individual tissues, it has been found necessary to determine the proportion of the total body weight accounted for by the different tissues. The following observations have been made:

Table 1. Showing the weight of certain tissues of the sheep foetus at different ages, and of one lamb and one adult sheep, expressed as a percentage of total body weight

	Age (in days)						Lamb	
Tissue	72	97	104	120	135	142–3	6 days	Adult
Whole skin	5·2	10	12·3	17·0	—	17·0	18·5	12·9
Shaved skin	5·2	10	10·1	—	—	11·0	12·0	3·1
Skeleton	21·9	29·0	27·0	23·0	—	23·4	25·0	10·6
Muscle	52·0	30·5	28·0	28·0	—	22·5	30·0	39·0
Liver	7·2	5·0	4·0	3·7	3·0	2·3	2·1	2·6
Gut (empty)	2·8	2·8	3·0	2·0	2·4	2·3	6·3	7·8
Lung	3·4	4·5	5·0	4·0	2·8	3·0	1·5	1·5
Brain	2·3	2·8	2·2	3·0	1·4	1·2	1·3	0·3
Heart	1·5	1·0	1·0	1·0	—	1·2	0·9	0·5
Kidney	1·3	1·0	1·0	1·0	—	1·1	0·6	0·5
Blood	5·0	5·0	12·0	3·0	—	3·5	6·3	4·9

The effect of diet on foetal development. By L. R. Wallace. *School of Agriculture, Cambridge*

Birth weight tells nothing of the effect of maternal diet on the various component parts of the foetus, which normally grow at widely differing rates (Jackson, 1909; Lowrey, 1911; Wallace, 1945). An important question is whether all parts and tissues are affected equally or differentially when foetal growth is retarded. Hammond (1943) has suggested that some parts are penalized more than others.

An investigation was conducted to decide this point. By feeding some ewes on a much restricted ration during the last 8 weeks of pregnancy, 144-day-old foetuses were obtained whose mean weight averaged only 57 % of those obtained from liberally fed ewes. Foetuses were also obtained at the 91st day of pregnancy, when the experimental treatments commenced. The data resulting from the dissection of these groups of foetuses were compared with those from well-fed ewes at 28-day intervals from service.

The results showed that, although all foetal parts from ill-fed mothers weighed less than corresponding parts from liberally fed dams, some tissues were more severely affected than others. Under conditions of restricted nutrition, nerve tissue competed for available nutrients more effectively than bone, and bone more effectively than flesh (muscle and fat). Growth of nerve tissue and bone was not depressed as much as that of the foetus as a whole, but growth of flesh more so.

Within the skeleton there was no, or at most only a slight, differential effect, so that in terms of individual bones, skeletons from the small 144-day foetuses had the same proportional composition as those of similar weight derived from younger better-grown foetuses.

As with the major tissues, so with the organs. For example, the alimentary tract was less affected than the heart, the heart less than the lungs and kidneys, and these in turn less than the thymus, spleen and liver.

From the foregoing it appears that the manner in which the weight increment made by the foetus during any particular period is distributed among the component parts of the body, will depend upon the extent to which the nutritive conditions then prevailing are limiting the rate of growth of the foetus. Implicit in this is the fact that foetuses that are small as a result of malnutrition of the mother are not mere miniatures of large ones of the same age; nor are they similar in composition to younger better-grown ones of the same weight.

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An integration of the total oxygen consumption of the sheep foetus, from estimations of the Q_{O_2}'s of individual tissues. By

A. CARLYLE. *A.R.C. Unit of Animal Physiology, Cambridge*

Although values have been reported for the oxygen consumption of the mammalian foetus in different species (Needham, 1931, 1942), the techniques employed are not regarded as entirely satisfactory. In order to check figures obtained in this laboratory for the sheep foetus at different ages (Barcroft, Kennedy & Mason, 1939) by an independent method, we have endeavoured to integrate the total oxygen uptake from estimations of the Q_{O_2}'s of individual tissue slices. The figures reported here (Table 1) were obtained with standard Warburg manometers, using as medium Ringer-phosphate—0·2 % glucose,

TABLE 1. Oxygen uptake of certain tissues of the sheep foetus at different ages, and of one lamb and one adult sheep, expressed as cu.mm./mg. dry wt./hr.

	Age (days)						
Tissue	78	99	112	130	144	Lamb 6 days	Adult
Skin	3·7	2·0	3·0	2·8	2·3	2·2	1·2
Muscle	2·4	3·1	2·0	1·0	0·7	0·6	1·2
Cartilage	3·1	2·4	0·8	0·6	0·8	0·9	0·3
Bone	—	0·1	0·1	0·1	0·1	0·05	0
Liver	10·8	7·5	6·8	6·4	7·3	5·8	3·6
Intestine	—	—	—	5·2	8·4	7·5	5·6
Stomach	—	4·7	2·1	4·3	4·4	4·9	0·9
Lung	2·3	2·2	3·5	4·3	2·8	5·2	3·0
Brain	—	3·4	6·1	5·8	8·3	13·6	2·9
Blood	—	0·2		0·2	0·1	0·2	0·3
Kidney	8·7	—		—	—	—	—

and a pure oxygen gas phase. Using values for dry-matter content and proportional weights of these tissues previously reported (Carlyle, 1945*a*, 1945*b*), we have calculated the expected total oxygen consumption (Fig. 1).

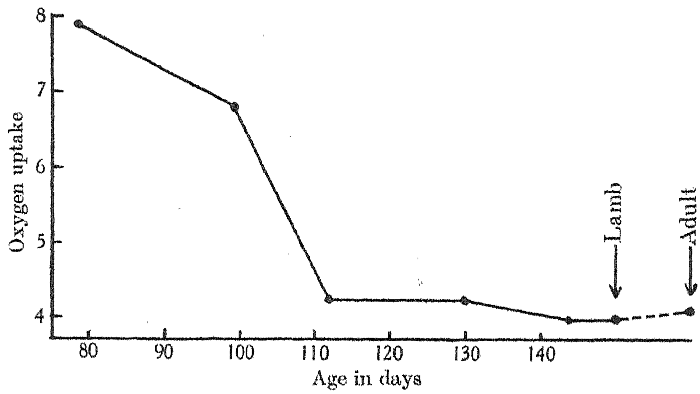

Fig. 1. Expected oxygen uptake of whole animal. (c.c./Kg./min.)

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Action of anti-coagulants on shed blood. By S. BRANDON STOKER and J. POLLARD (introduced by D. BURNS). *Department of Physiology, King's College, Newcastle-upon-Tyne*

The action of anti-coagulants on shed blood depends in great measure on three circumstances: (1) the initial clotting time (I.C.T.) of the blood, (2) the amount of anti-coagulant added, and (3) the chemical nature of the anti-

coagulant, i.e. whether it is basic like toluidin blue, or acidic like chlorazol fast pink or heparin. With the usual comparatively large additions of anti-coagulant, it is difficult to differentiate between the actions of the various anti-coagulants, but when the amounts added are small, it is clear that basic and acidic substances act differently.

(*a*) *Basic dyes.* The addition of toluidin blue in amounts between 1·2 and 2·5 mg./100 ml. blood, whatever the I.C.T. of the blood, causes the blood to clot between 5 and 6 min. If the I.C.T. is already 5–6 min., toluidin blue has no apparent action on it. Reduction to 0·1–0·3 mg./100 ml. of the amount of toluidin blue added produces a more marked effect on blood of long I.C.T. as shown in Table 1.

TABLE 1.

	7	$7\frac{1}{2}$	$8\frac{1}{2}$	$9\frac{1}{2}$	I.C.T. blood in min.
Toluidin blue 0·1 mg./100 ml. blood	$+4\frac{1}{2}$	$+3$	0	$-3\frac{1}{2}$	min. change in C.T.
0·2 ,,	$+2\frac{1}{2}$	$+1\frac{3}{4}$	$-\frac{1}{2}$	-3	min. change in C.T.
0·3 ,,	$+1\frac{1}{2}$	$+2$	$-\frac{1}{2}$	-4	min. change in C.T.

The greatest action is given by the smallest addition which while producing no action on blood with I.C.T. of 8–9 min. does not tend to convert blood of other C.T. to 8–9 min.

This 'irregular sequence' is also shown by the acidic substance heparin at certain concentrations.

(*b*) *Acidic anti-coagulants.* We find that heparin (B.D.H. 120 i.u./mg.) in quantities of 0·1–0·75 mg./100 ml. of blood, has a maximum anti-coagulant action on blood of I.C.T. 5 min. With lengthening I.C.T. the anti-coagulant effect diminishes until with blood of I.C.T. 8 min. or over the effect is actually to shorten C.T. Fischer (1931), using fluids other than fresh blood, showed that very small amounts of heparin have a greater anti-coagulant action than somewhat larger quantities. We find that this 'irregular sequence' is at a maximum around 0·03 mg./100 ml. of blood. The quantities of toluidin blue and heparin giving this effect are approximately chemically equivalent amounts. In contrast to the results with toluidin blue, where the individual C.T. plotted against I.C.T. lie amost wholly (85 %) within narrow diagonal bands, there is with heparin a characteristic scatter which is greatest at lower I.C.T. With blood of 5 min. I.C.T., the addition of 0·25 mg. of heparin per 100 ml. of blood may lengthen C.T. by anything between 1 and 6 min. This scatter is in conformity with the results obtained by De Takats (1943), who found that the administration of heparin (10 mg. i.v.) might either have no effect on C.T. or might lengthen it by 1–4 min. As Quick (1944) says: 'The key to the physiological action of heparin appears to be its strongly acidic property by virtue of which it forms stable salts with many proteins.' That is, the activity of heparin will be modified by the utilization of some of the heparin to form complexes with any proteins in the blood more basic than thrombin (Fischer, 1935). A rough measure of the amount of such proteins present is the extent of the scatter. Taking the upper and lower limits of the scatter for blood of I.C.T. 5 min. to be 1 and 6 min., it would require the addition of 0·2 mg./100 ml. of heparin to raise the C.T. from the lower to the higher limit of scatter. Other factors, e.g. alkali reserve (Yates, 1928), certain lipoids and the albumin-globulin ratio (Ziff & Chargaff, 1940), may play a part in this. Work is proceeding on the study of C.T. in various clinical conditions known to produce alterations in some of these anti-heparin factors.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY

1 *December* 1945

Human centrifuge and studies of black-out. By E. J. BALDES, C. F. CODE, E. H. LAMBERT and E. H. WOOD. *Acceleration Laboratory, Mayo Aero Medical Unit, Mayo Clinic and Mayo Foundation, Rochester, Minnesota, U.S.A.*

Human centrifuges have been built in an attempt to reproduce under controlled conditions in a laboratory the accelerations to which aviators may be exposed during sharp turns, pull-outs, spins or other manoeuvres in aircraft. Two such devices have been built in Rochester: a small pilot model suitable for animals in 1941, and a larger one for human work in 1942. Both operate in a similar manner. They consist essentially of a carriage or superstructure below which one or more heavy flywheels rotate. The heavy flywheels, weighing in the case of the human centrifuge a total of 40 tons, provide the energy for the rapid development of accelerative forces in the superstructure. The superstructure may be quickly or slowly set in motion by clutching to the rotating flywheels, and may be stopped rapidly or slowly by declutching and applying a brake.

This device has allowed accurate studies of man's reactions to acceleration. Most complete data have been obtained during exposures to so-called positive g in which the reactive force is applied in the direction from head to seat in the seated subject. During such exposures there is a definite sequence of physiologic events. These fall sharply into two distinct periods—a period of progressive failure followed by a period of compensation. During the period of progressive failure the pulse rate progressively increases, the blood content of the ear is progressively reduced, pulsations of blood in the ear may be gradually reduced or abruptly lost, the blood pressure at head level falls, and symptoms if they are to occur become evident. The greater the accelerations, the greater the magnitude of these changes. The progressive failure is terminated as a rule by a compensatory reaction which usually becomes effective about 6–12 sec. after the onset of acceleration. The period of compensation then occurs, during which the blood pressure rises, the ear pulse improves, the amount of blood in the ear increases, the increase in heart rate is checked and the pulse may slow. If these changes are sufficiently effective, recovery from symptoms will occur.

Upon the basis of these physiologic changes a bio-assay procedure has been developed which allows the accurate determination of man's g tolerance and of the protective value of any device or procedure designed to offset the

deleterious effects of positive g. The assay procedure is based upon the recognition and determination of the g level at which various subjective symptoms occur (dimming of vision, loss of peripheral vision and complete loss of vision) and upon the measurement of certain objective changes in the subject (loss of blood from the ear, reduction or loss of blood pulsations in the ear, degree of pulse-rate increase, the magnitude of blood-pressure changes) during exposure to various amounts of acceleration with and without the protective device or procedure.

The assay has allowed an orderly and quantitative approach to the problem of protecting the aviator against the effects of positive acceleration, and has led to the development of various pneumatic suits and other protective devices and procedures which effectively reduce the period of progressive failure and maintain the aviator in good physiological condition at accelerations which would ordinarily produce black-out or unconsciousness.

Presso-receptors of the carotid sinus and respiration. By C. Heymans and R. Pannier. *Institute of Pharmacology, University of Ghent*

In previous publications (Heymans, 1928, 1929; Heymans & Bouckaert, 1930; Heymans, Bouckaert & Regniers, 1933; Winder, 1938) it has been shown that blood-pressure changes in the carotid sinus induce respiratory reflexes; increase of blood pressure in the carotid sinus provokes reflex inhibition of respiration, even apnoea, while decrease of blood pressure induces reflex respiratory stimulation. These respiratory reflexes of carotid sinus origin are due, as shown by means of several experimental methods, to the influence of the pressure changes acting on the presso-receptors of the carotid sinus. Euler & Liljestrand (1936, 1937), Rudberg (1938, 1940), Bjurstedt & Euler (1942), Bjurstedt & Hesser (1942), Gernandt, Liljestrand & Zotterman (1945) concluded from their experiments that these respiratory reflexes are not due to an action of the blood pressure on the presso-receptors, but to an influence of the changes of the blood supply on the activity of the chemo-receptors of the carotid sinus area.

In dogs anaesthetized with chloralosane, the vagi-aortic nerves are cut, the efferent branches (internal and external carotid and occipital arteries) of both carotid sinuses are tied at their origin, care being taken not to sever the presso-sensitive innervation (Heymans *et al.* 1933; Bouckaert & Pannier, 1942). The cephalic ends of the common carotid arteries are connected with a pressure device. By means of this technique, the chemo-receptors which are located in the ganglion caroticum are excluded, and the hydrostatic pressure may be increased or decreased in the isolated but only presso-sensitive innervated carotid sinus. Increase of the hydrostatic pressure induces a reflex inhibition of the respiratory centre, even a reflex apnoea, while decrease of pressure produces a reflex stimulation of the respiratory centre, a reflex hyperpnoea. These experimental facts do not exclude, however, the reflex influences on the respiratory centre induced by the action of blood-flow variations on the chemo-receptors of the carotid sinus (ganglion caroticum)

as is well shown by Bjurstedt & Euler (1942), Bjurstedt & Hesser (1942), Euler & Liljestrand (1936, 1937), Euler, Liljestrand & Zotterman (1939), and Gernandt, Liljestrand & Zotterman (1945). Thus blood pressure acting on the presso-receptors of the carotid sinus and blood flow acting on the chemo-receptors of the ganglion caroticum have both a reflex influence on the activity of the respiratory centre.

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The production of an adrenaline-like substance by the heart.

By R. J. S. McDowall. *King's College, London*

In June 1944 it was reported to the Society that minute doses of acetylcholine stimulated the heart and sensitized it to adrenaline. In November it was shown (McDowall, 1944) that the effect could be shown with larger doses after atropine and abolished by ergotoxine. In July 1945, Hoffmann, Hoffmann, Middleton & Talesnik confirmed these results and described the liberation into the coronary perfusion fluid after atropine and acetylcholine of a substance claimed to be adrenaline.

It has now been found that finely minced heart of rabbit or preferably cat treated with acetylcholine produces a substance which relaxes and reduces the movements of the atropinized rabbit intestine, solutions of acetylcholine and saline extracts having no such effect. It has not been found possible to find the substance in all hearts, but great individual variability was also found in regard to the stimulation of the perfused hearts. The active substance may be adrenaline, but it may be acetylcholine in an altered form.

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Effect of magnesium ions on the enzymic formation of acetylcholine.
By W. Feldberg and Catherine Hebb.* *Physiological Laboratory, Cambridge*

If a rat's brain is ground with acetone and the acetone powder extracted with 0·9 % NaCl a solution is obtained which synthesizes, on addition of adenosinetriphosphate, choline, KCl, cysteine and citrate, large quantities of acetylcholine; this synthesis occurs both under aerobic and anaerobic conditions. It is but slightly affected by the addition of magnesium ions (Feldberg & Mann, 1944, 1945, 1946). The enhancing effect of magnesium ions becomes prominent, however, if the enzyme solution extracted from the acetone-dried brain powder is first extensively dialysed against 0·9 % NaCl and only then used for experiment. Thus, for instance, a dialysed extract synthesized in 1 hr. at 37° C. only 30 μg. acetylcholine per g. acetone powder whereas the same extract synthesized on addition of 0·002 *M* $Mg^{\cdot\cdot}$ 600 μg./g. powder. In another similar experiment on addition of 0·0004 *M* $Mg^{\cdot\cdot}$ 400 μg./g. were formed. It was further found that Mn 'can replace Mg'.

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Further observations on the causes of a diuresis during hydropenia. By G. R. Hervey, R. A. McCance and R. G. O. Tayler

In a recent paper, McCance (1945) has shown that at low minute volumes maximum concentrations of urea and chlorides do not occur simultaneously in human urine. This was attributed, we believe correctly, to the osmotic limitations of the kidney. When, however, a diuresis was produced by administering hypertonic salt to a dehydrated person the osmotic pressure of the urine fell, whereas the concentration of chlorides remained at or near their maximum. The conclusion was drawn that at higher minute volumes the osmotic pressure of the urine did not prevent its further concentration, but that reabsorption of water in the distal tubules was limited by the concentration of chloride reached within them.

We have, therefore, attempted to produce maximum concentrations of urea and chlorides simultaneously at high urine flows by taking urea as well as salt, and the purpose of this communication is to report that we have failed to do so, although the previous experimental results have been confirmed. At any given urine flow over about 3 c.c./min., taking urea as well as salt has depressed the concentration of urinary chlorides and raised very little, or not at all, the total osmotic pressure of the urine. We have, therefore, come to the conclusion that the reabsorption of water in the distal tubules is not limited by the concentration of chlorides within them reaching a 'maximal' value, but that there is a limiting osmotic pressure at all urine flows and that this falls as the minute volume rises.

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Oesophageal activity in men during water deprivation.

By W. S. S. Ladell. *National Hospital, Queen Square, London, W.C.* 1

Oesophageal activity was estimated by recording optically the changes in pressure in a small oesophageal balloon inflated with 10 c.c. of air at atmospheric pressure. In two subjects records were taken when they were fully

hydrated and again when they were in water debt. In the normal state the oesophagus was practically quiescent at the level of the arch of the aorta but more active at each end, particularly near the diaphragm. In the 'aortic' region there were occasional spontaneous contractions sometimes as powerful as those accompanying the act of swallowing. The subjects were unconscious of these contractions.

Records taken after 36 hr. water deprivation showed more activity throughout the whole length of the oesophagus on swallowing the balloon and for some time after. But when the subjects had swallowed dry or wet a few times, this activity died away and the oesophagus again became quiescent. The increased activity shown on first swallowing the balloon could be interpreted as due to an increased irritability of the dry oesophagus. This irritability became more marked the longer the duration of deprivation of water, but the oesophagus never failed eventually to become quiescent. Muller's (1920) thesis that in thirst there is a continuous turbulent activity of the oesophagus has not been confirmed.

Both the pressure changes and the speed of the contraction wave accompanying swallowing were the same when the subjects were in water debt as when they were hydrated.

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